MARINE BIOLOGICAL LABORATORY

FORMULAE AND METHODS IV

WOODS HOLE, MASSACHUSETTS

FORMULAE AND METHODS IV.

OF THE

MARINE BIOLOGICAL LABORATORY

CHEMICAL ROOM

Gail M. Cavanaugh, Editor

PREFACE TO FOURTH EDITION

Our Formulae and Methods Manual has been prepared from information acquired by our Chemical Room Staff over the course of more than 25 years. The first, second, and third editions were prepared and edited by Oscar W. Richards. The first edition was published as a supplement to The Collecting Net on August 30, 1930. It contained 12 pages of information on biological solutions, stains, buffers and photographic solutions. The second edition, containing additional information on the above subjects, was published on August 27, 1932. The Formulae and Methods Manual was completely revised and published as the third edition by Oscar W. Richards in 1936.

During the past 15 years more information regarding artificial sea water, buffers, and other biological solutions and formulas has been obtained by our staff. This information has resulted in publication of The Fourth Edition. This edition contains a complete revision of the sections on buffers, artificial sea water, and photographic solutions. Several new tables have been added. It is interesting to note that the material on biological stains has remained practically unchanged during the years. A table of contents has been added for quick references.

The editor wishes to express his thanks to J. D. Ostrow for his work on Chapters 2, 3, 7, 8 and 9; to Mary Kapp for her work on stains; to J. B. Russell for his assistance on buffers; to A. Bickel for his work on primary and secondary standards; and to E. B. Harvey for her work on the artificial sea waters.

G.M.C., 1954

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CHAPTER I.

GENERAL INFORMATION

PURITY OF CHEMICALS:

Several grades of many of the chemicals are kept in stock and care must be used in issuing chemicals so that the proper quality is furnished to the investigator. If there is any doubt as to the quality or quantity requested, consult with the person in charge before filling the order.

Every precaution must be taken to prevent contamination of the U.S.P. and Reagent grades. The necks and caps of all reagent bottles should be free from dust before the bottle is opened. Spatulas are not to be introduced into the reagent stock bottles unless necessary, and then only after they have been thoroughly cleaned and dried. Material removed is not to be returned to the stock bottles of the higher grade chemicals. When an amount is issued in other than the original container the label must contain the name of the chemical, the name of the manufacturer, the grade and the lot number. Metal spatulas are not to be used in handling mercuric chloride, iodine, silver nitrate, and other corrosive chemicals. When in doubt use glass or porcelain spoons. The weighing papers are to be used only once and all tools immediately washed and placed where they will drain and dry. Any chemical that is spilled is to be cleaned up immediately and put into the proper receptacle.

The commercial or technical grade is satisfactory for many purposes such as freezing mixtures and cleaning fluids. U.S.P. chemicals have been prepared to meet the standards of the United States Pharmacopoea (qv) and while suitable for medicinal use may contain other impurities harmless for this purpose. The so-called chemically pure (C.P.) grades are more or less pure but as there are no generally accepted standards for these grades, the purity will vary with different lots and samples from different manufacturers. This grade is useful when the highest purity is not required.

The purest chemicals commercially obtainable are further purified and are accompanied with an analysis indicating the tolerances or limits of certain impurities contained. The standards are based on those established by the American Chemical Society or given in Murray, Standards and Tests for Reagent Chemicals, Van Nostrand. No information is available for other impurities not tested for and when the investigator is in doubt it is essential that he make the necessary tests or further purify the chemicals in accordance with his requirements. Since the

analyses are only limits of tolerance they are of little use in comparing different brands of chemicals which may contain varying amounts of other impurities.

The purest grades include Merck's "Reagent Grade", Malinckrodt's "Analytical Reagent", Eimer and Amend's "Tested Purity", Baker's "Analyzed", etc.

Reagent grade and special chemicals are to be issued only when this grade of purity is requested on the order.

The confusion of different grades of materials is to be guarded against. For example, do not issue immersion oil for clearing oil just because both are different kinds of cedar oil. Certain of these confusing substances are specially marked. In the case of expensive materials issue no more than the amount marked on the bottle unless permission of the person in charge is given for a greater amount.

SOLUBILITIES:

If a special solution calls for a large amount of a material consult the table of solubilities, Chapter 5, or for stains, Chapter 4, to see if solution is possible. The Merck Index, Chemical Rubber Handbook of Chemistry and Physics, Lange's Handbook of Chemistry, International Critical Tables, and the Dictionary of Chemical Solubilities are excellent reference books concerning solubilities of compounds. If in doubt, consult with the person in charge before attempting the preparation. This may avoid an error and the wastage of much material. Chloretone, in particular, cannot be made stronger than 0.6% in water at room temperature.

ACCURACY AND DECIMALS:

Note that accuracy is relative. An error of 0.1 gram in 500 grams is only a 0.02% error, while the same error in 1 gram is a 10% error. Use the proper scales or balance for the particular job to be done. If there is any question as to the accuracy required in an order, consult with the investigator placing the order or with the person in charge. When a decimal is to be placed on the label of any preparation conform to the following rule: Write the decimal to the number of places known to be correct, and only one doubtful figure. This same rule can be applied to figures where the known value does not extend to decimals by writing one doubtful figure and the rest zeros.

ACCURACY AND ERRORS:

Absolute errors $x, -x$ are deviations from the correct values and their sign is important for correct statement. They are expressed as

correct to two decimals, or to the nearest million, etc. Absolute errors are more important in addition and subtraction: e.g., in a column of figures the absolute errors in the third place of a sum or a difference may be great enough to make the second place unreliable. Relative errors $(x_1 - x)/x$ are connected with the number of significant figures and are usually expressed as percentages. These errors are important in multiplication and division. In a product or quotient the number of significant figures is equal to the number in the weakest factor. Many solutions need not be prepared more carefully than 5% while others must be made with care to insure sufficient accuracy. If in doubt as to the precision required consult with the investigator or with the person in charge. This information and that given above is to be used as a guide by the staff in the use of the equipment in the Chemical Room.

ALCOHOL DILUTION:

For ordinary histological work special strengths of alcohol may be prepared by taking the number of milliliters of 95% alcohol equal to the strength desired in a graduate and adding enough distilled water to make 95 ml. (Example: to prepare 60% take 60 ml. of 95% alcohol and add 35 ml. of distilled water making 95 ml. of the strength of the alcohol used.)

CLEANING METHODS:

Scrubbing with a 2% solution of alconox and hot water followed by a liberal rinsing with tap water will remove most chemicals. A small amount of trisodium phosphate applied with the fingertips or with a small brush will remove pencil markings, most greases, xylene, and films of paraffin. No abrasives should be used in cleaning volumetric glass ware. When cleaning solution (sulfuric acid-dichromate) is used continued rinsing is necessary. Ten rinsings with water, seven of tap and three of distilled, are necessary for adequate removal of the dichromate from the surface of the glass. Since chromic acid is toxic to living organisms, this is important. For many purposes 1 part concentrated nitric acid to 9 parts water is superior to chromic acid as the nitric acid oxidizes organic material without leaving an adsorbed residue on the glass. Aqua regia will remove what can be removed by cleaning solution and will wash off completely with tap water. To clean staining jars use a little dilute hydrochloric acid. For the few dyes not removed by this, use a strong solution of sodium hydroxide. Another useful cleaning fluid is 1-5% trisodium phosphate. Rubber stoppers may be cleaned by boiling in dilute sodium hydroxide, then rinsing with

water, followed by boiling in dilute hydrochloric acid and finally thoroughly rinsing with water.

GLYCINE or GLYCOCOLL is an amino acid used medicinally and is not to be confused with the poisonous photographic developer glycin, (p-hydroxyphenylaminoacetic acid).

A MOLAL SOLUTION (m) contains one gram-molecular weight dissolved in 1000 grams of solvent. For ordinary aqueous solutions 1 ml. of water is used as 1 gram. For other solutions calculate according to density at the temperature used.

A MOLAR SOLUTION (M) contains one gram-molecular weight in one liter of solution. Dissolve the material in less than one liter and make up to one liter in a volumetric flask.

A NORMAL SOLUTION (acidimetry or oxidimetry) contains one equivalent of the active reagent in grams in one liter of solution. The equivalent value of any reagent will depend upon the conditions under which the reagent is employed. It may or may not be the same as a molar solution.

PERCENTAGE SOLUTIONS: Percent means parts in one hundred parts. These solutions may be made up according to weight, volume, or any combination of these. For example, a 3% solution of KCl contains 3 grams of the salt in 100 grams of solution, or in 97ml. of water. A 3% solution can be made up in any one of three ways: (a) 3 grams of KCl in a total volume of solution of 100 ml.; (b) 3 grams in 100 ml. of water; (c) 3 grams in 97 grams of water. The third method will give precisely a 3% solution. The first two methods do not give a 3% solution, but for concentrations of 3% or less the error is too small to be of significance. For percentages greater than 3% it is best to prepare the solution on the basis of weight. For example, a 40% solution of NaOH is made by adding 40 grams of NaOH to 60 grams of water. Some substances, e.g., alcohol, vary in strength according to percent by weight or volume. Percentage solutions (by weight) may be prepared with the solution balance. Place the bottle, or bottle and funnel, on the pan and balance by means of the weight on the ungraduated beam. Set the weight on one of the graduated beams and weigh out the solute, then set for the amount of the solution and add the solvent until the scale is balanced. The beams are graduated to facilitate the preparation of percentage solutions but the balance may be used to advantage for the preparation of other solutions.

The dilution of percentage solutions (aqueous solutions by weight) can be accomplished easily by taking the number of milliliters

(or multiples thereof) of the stock solution equal to the strength solution desired and adding enough distilled water to make the total number of milliliters equal to the strength of the stock solution. Examples, (a) to prepare 7.1% from 18% stock solution use 7.1 ml. of the stock plus 10.9 ml. of water which makes a total of 18 ml. (b) to obtain a 0.02% solution from a 0.4% stock solution use 1 ml. of stock (50 x 0.02) and 19 ml. of water (50 x 0.38) making 20 ml. (50 x 0.02 + 50 x 0.38) = (50 x 0.4) of the required solution.

In general, remember that in diluting solutions the volume of the concentrated solution times its concentration is equal to the volume of the dilute solution times its concentration, or

$$Vol_1 \times Conc_1 = Vol_2 \times Conc_2$$

To make a 3/8 M solution from a 1M solution, take three parts of the 1M solution and dilute to eight parts. For example, to make 500 ml. of a 3/8 M solution of NaCl from a 1M solution take 3/8 of 500 or 186.5 ml. of 1M NaCl and dilute to 500 ml. To make 50 ml. of a 1:10,000 solution requires 1:10,000 of 50 or 0.005 grams of the active ingredient made to 50 ml. with water.

PROOF is the scale used for measuring the strength of alcohol. Absolute alcohol is 200 proof; and a mixture containing 50% alcohol by volume is 100 proof (U.S.A.)

A proof-gallon contains an amount of alcohol equal to that in a gallon of proof spirit (100 proof). A gallon of proof spirit is one-half alcohol. Wine gallons multiplied by 1.9 equals proof gallons.

DELIQUESCENT CHEMICALS. Bottle tops of the following chemicals should be dipped in paraffin.

Acetamide	Beryllium sulfate	Magnesium chloride
Acid arsenic	Calcium bromide	Magnesium nitrate
Acid chromic	Calcium chlorate	Manganese chloride
Acid citric	Calcium chloride, anhyd	Manganese sulfate
Acid monochloracetic	Calcium chloride, cryst	Mercuric nitrate
Acid silicotungstic	Calcium nitrate	Potassium acetate
Acid trichloracetic	Calcium oxide	Potassium carbonate
Aluminum chloride	Cobalt sulfate	Potassium thiocyanate
Aluminum nitrate	Ferric chloride	Sodium arsenate, cryst
Ammonium acetate	Ferric nitrate	Sodium chlorate
Ammonium fluoride	Ferrous chloride	Sodium hypophosphite
Ammonium thiocyanate	Iodides (most forms)	Sodium selenate
Barium bromide	Lithium bromide	Sodium sulfide
Barium chloride	Lithium chloride	Sodium sulfite, anhyd.
Beryllium chloride	Lithium salicylate	Sodium thiocyanate
Beryllium nitrate	Magnesium bromide	Starch
		Zinc chloride

CHAPTER II.

GENERAL FORMULAE

NOTE: Use distilled water in all solutions.

ADAMKIEWICZ REAGENT: (for proteins). Concentrated sulfuric acid 1 volume, glacial acetic acid 2 volumes. Heat substance with this reagent. Reddish-violet color denotes proteins.

AGAR: 2% may be used to solidify various solutions.

ALCOHOL, ACID: 70% alcohol 99 ml., conc. hydrochloric acid 1 ml.

ALCOHOL, ALKALINE: 70% alcohol 99 ml., 0.02 grams sodium bicarbonate.

AMANN'S LACTOPHENOL: Phenol 20 grams, lactic acid (1.21 sp.gr.) 16.5 ml., glycerine 32 ml., water 20 ml. For herbarium specimens, soften first in 1:10 lactophenol and then to pure lactophenol. For algae use, lactophenol 5 ml., water 95 ml., cupric chloride and cupric acetate each 0.2 grams. For mounting fungi add to the lactophenol 0.05 grams cotton blue.

AMMONIACAL SILVER SOLUTION: Dissolve 13 g. of silver nitrate in about 250 ml. of water, add enough conc. ammonium hydroxide to re-dissolve the precipitate which forms upon the first addition of the NH_4OH and make the volume up to 500 ml. with water.

ANILINE WATER: Shake 4 ml. of aniline in 90 ml. of distilled water for at least 15 minutes. Filter through a wet filter. Enough alcohol may be added to make it 20% alcohol, if a weakly alcoholic solution is desired.

BARFOED'S SOLUTION: (test for dextrose in presence of maltose) 13.3 g. cupric acetate in 200 ml. water, and 5 ml. of 38% acetic acid.

BELAR'S SOLUTION: Water 100 ml., sodium chloride 0.9 g., potassium chloride 0.02 g., calcium chloride 0.02 g., sodium bicarbonate 0.02 g. To this 0.25 to 0.5 g. glucose may be added.

BENECHE'S NUTRIENT SOLUTION: (Algae-bluegreen) Water 1 liter, ammonium nitrate 0.2 g., calcium chloride 0.1 g., potassium phosphate (dibasic) 0.1 g., magnesium sulfate 0.1 g., 1% ferric chloride 1 drop. For use dilute 2-9 times as required. (Courtesy Dr. Navez)

BENEDICT'S SOLUTION - QUALITATIVE: Cupric sulfate 17.3 g., Sodium citrate 173 g., sodium carbonate 100 g., water 1000 ml. Dissolve the sodium citrate and carbonate in 800 ml. water (filter if necessary). Dissolve the cupric sulfate in 100 ml. Add the cupric sulfate slowly to the citrate-carbonate solution with constant stirring and dilute to 1 liter.

BENEDICT'S QUANTITATIVE SUGAR REAGENT: Cupric sulfate 18 g., sodium carbonate (1/2 weight of anhydrous salt may be used) 200 g., sodium or potassium citrate 200 g., potassium thiocyanate 125 g., potassium ferrocyanide (5% solution) 5 ml., water to make up volume to 1000 ml. With the aid of heat dissolve the carbonate, citrate, and thiocyanate in enough water to make 800 ml., and filter if necessary. Dissolve the cupric sulfate separately in 100 ml. and mix slowly with the other solution. 25 ml. of the reagent are reduced by 50 mg. of glucose.

BRODIE'S SOLUTION: Water 500 ml., sodium chloride 23 g., sodium chlorate 5 g., 1% (aq.) methylene blue 3 ml., thymol 0.1g.

CARBO-XYLOL: 1 part liquified phenol (see below), and 3 parts of xylene.

CARLSBAD SALTS, SYNTHETIC: (Sprudelsalz) Sodium sulfate 11 g., sodium bicarbonate 10 g., sodium chloride 9 g., potassium nitrate 19 g., potassium sulfate 1 g.

CEMENT: Beeswax 58%, rosin 29%, Venetian turpentine 13%.

CHALKLEY'S MEDIUM: Water 1000 ml., sodium chloride 0.1 g., potassium chloride 0.0004 g., calcium chloride 0.006 g.

CHLORETONE: Chlorbutanol 0.6 g. soluble in 100 ml. water 20° C.

CLARK'S FLUID: (for insect tissue culture) Water 200 ml., sodium chloride 1.3 g., potassium chloride 0.028 g., calcium chloride 0.024 g., sodium bicarbonate 0.02 g., monobasic sodium phosphate 0.002 g.

CLEANING SOLUTION: Dissolve 60 - 65 g., of sodium or potassium bichromate by heating in 30 - 35 ml. of water. Cool and slowly add concentrated sulfuric acid to make a liter of solution.

CLERICI'S SOLUTION: 10 ml. water, 50 g. thallium (ous) malonate, 50 g. thallium (ous) formate, 0.1% sodium tauroglychocholate. Keep cool and filter. Density about 4.

CZAPEK'S MEDIUM: (for molds) Sucrose 30 g., sodium nitrate 2 g., dibasic potassium phosphate 1 g., magnesium sulfate 0.5 g., potassium chloride 0.5 g., ferrous sulfate 0.01 g., water 1000 ml.

FEHLING'S SOLUTION: Solution I. Cupric sulfate 34.65 g. in 500 ml. water. Solution II. Potassium hydroxide 125 g., Rochelle salts (sodium potassium tartrate) 173 g., water to make 500 ml. Solutions mixed in equal volumes for use.

FIESER'S FLUID: Water 100 ml., sodium hydrosulfite 16 g., sodium hydroxide 13.3 g., sodium anthraquinone-B-sulfonate 4 g.

FILM PRESERVATIVE: (acetate base) Camphor 4 g., menthol 4 g., oil eucalyptus 8 g., glycerine to make 125 ml.

GLYCERINE JELLY: Water 60 ml., gelatin 10 g., glycerine 70 ml., phenol (cryst.) 0.25 g. Soak gelatin 30 minutes in the water, dissolve with gentle heat. Add glycerine and phenol and stir until homogeneous. Store in wide mouth bottle.

GRAMS IODINE: Water 300 ml., potassium iodide 2 g., iodine 1 g. Mix KI and I_2 in a few drops of water. When dissolved add the remaining quantity of water.

GREEN FILTER SOLUTION: Water 300 ml., cupric sulfate 35 g., potassium dichromate 3.5 g., conc. sulfuric acid 1 ml.

GROUND GLASS SUBSTITUTE: Ether 240 ml., sandarach 12 g., mastic 2.6 g., benzene 160 ml.

HAINES SOLUTION: Water 170 ml., cupric sulfate 2 g., potassium hydroxide 6.7 g., glycerine 15 ml.

HAYEM'S SOLUTION: (for microscopic examination of blood) Water 1000 ml., mercuric chloride 2.5 g., sodium sulfate (anhyd) 25 g., sodium chloride 5 g.

HEAT ABSORBING FLUID: 2% aqueous calcium chloride.

HEAT ABSORBING FLUID: Water 1000 ml., Mohr's salt (ferrous ammonium sulfate) 200 g. Dissolve and filter. If not clear, add 1.7 ml. conc. sulfuric acid.

HOLTFRETER'S SOLUTION: Water 1000 ml., sodium chloride 3.50 g., potassium chloride 0.05 g., calcium chloride (anhyd) 0.10 g., sodium bicarbonate 0.20 g. When this is to be sterilized do not add the bicarbonate until ready to use.

IODINE, TINCTURE: U.S.P. 70% Alcohol 1000 ml., iodine 70 g., potassium iodide 50 g.

IODINE, WATER: Water 1000 ml., iodine 0.2 g.

KEEFE'S SOLUTION: (for preserving green organisms) Alcohol 50% 90 ml., formaldehyde 5 ml., acetic acid (glacial) 2.5 ml., glycerine 2.5 ml., cupric chloride 10 g., uranium nitrate 1.5 g.

KLOTZ SOLUTION: (for preservation of invertebrate animals) Chloral hydrate 50 g., Carlsbad salts (see above) 50g., formalin 100 ml., water to make 1000 ml.

KNOP'S SOLUTION: Water 1000 ml., calcium nitrate 1 g., potassium chloride 0.25 g., magnesium sulfate 0.25 g., monobasic potassium phosphate 0.25 g., ferric chloride trace.

KNOP'S SOLUTION: (modification) Water 1000 ml., potassium nitrate 1 g., calcium sulfate 0.5 g., magnesium sulfate 0.5 g., calcium phosphate 0.25 g., ferrous phosphate 0.25 g.

LOCKE'S SOLUTION: (for warm blooded animals) Water 1000 ml., sodium chloride 9 g., calcium chloride (anhyd) 0.24 g., potassium chloride 0.42 g., sodium bicarbonate 0.2 g., dextrin 2.5 g.

LUGOL'S IODINE: Water 100 ml., iodine 4 g., potassium iodide 6g. Mix KI and I_2 with a few drops of the water. When dissolved, add the remaining quantity of water.

MAYER'S ALBUMEN FIXATIVE: Separate the white albumen and cut it with scissors to break up the large masses. Filter through coarse filter paper and add an equal volume of glycerine. A crystal of thymol acts as a preservative.

MASSART'S MOUNTING MEDIUM: Water 100 ml., glycerine 16 ml., chloral hydrate 100 g., gum arabic 50 g. After fixing in distilled water for 24 hours, then 24 hours in 50% chloral hydrate followed by 24 hours in 100% chloral hydrate and then mounted in the above solution. (Courtesy Dr. Navez)

MILLON'S REAGENT: (for proteins and nitrogenous compounds) Mercury 10 g., concentrated nitric acid 15 ml. Dissolve the mercury in the acid, then dilute the solution with 2 volumes of water. Let stand 24 hours and decant. Gives a red color with proteins.

MIQUEL'S SOLUTION: I (culture fluid for diatoms) A. Water 100 ml. magnesium sulfate 10 g., sodium chloride 10 g., sodium sulfate 5 g.,

ammonium nitrate 1 g., potassium nitrate 2 g., sodium nitrate 2 g., potassium bromide 0.2 g., potassium iodide 0.1 g. B.* Water 80 ml., sodium phosphate 4 g., calcium chloride (anhyd) 4 g., conc. hydrochloric acid 2 ml., ferric chloride 2 g.

*Make as follows: To the sodium phosphate dissolved in 40 ml. of water are added, first 2 ml. of HCl, then 2 grams of ferric chloride and then 4 grams of calcium chloride dissolved in 40 ml. of water, taking care to shake the mixture. There will be a brown precipitate of ferric oxide on adding the last solution. It should be separated from the liquid before using. Forty drops of solution A and 10 - 20 drops of solution B are added to each 1000 ml. of sea water sterilized by keeping at 70°C for about 20 minutes.

MIQUEL'S SOLUTION: II (May be added to A and B) Sodium silicate $\cdot 9\ H_2O$ 5 g., water 100 ml.

MIQUEL'S SOLUTION: III (Allen's modification) A. Water 100 ml., potassium nitrate 20.2 g. B. Water 80 ml., dibasic sodium phosphate 4 g., $CaCl_2 \cdot 6\ H_2O$ 4 g., ferric chloride (melted) 2 ml., conc. HCl 2 ml. For use, add 2 ml. of solution A and 1 ml. of solution B to each 1000 ml. of sea water.

MOORE'S SOLUTION: Water 1000 ml., ammonium nitrate 0.5 g., monobasic potassium phosphate 0.2 g., magnesium sulfate 0.2 g., calcium chloride (anhyd) 0.1 g., ferric sulfate trace.

NAEGELI'S SOLUTION: (culture medium for fungi) Water 1000 ml., dibasic potassium phosphate 1 g., magnesium sulfate 0.2 g., calcium chloride (anhyd) 0.1 g., ammonium tartrate 10 g.

NESSLER'S REAGENT: (Block and Benedict from Hawk and Bergein) Mercuric iodide 100 g., potassium iodide 70 g., sodium hydroxide 100 g. Place 100 g. mercuric iodide and 70 g. of potassium iodide in a liter volumetric flask and add about 400 ml. of water. Rotate until solution is complete. Now dissolve 100 g. of sodium hydroxide in about 500 ml. of water, cool thoroughly and add with constant shaking to the mixture in the flask, and make up with water to the liter mark. If a precipitate forms, decant the supernatant liquid and use.

PASTEUR'S SOLUTION: Potassium phosphate 2 g., calcium phosphate 0.2 g., magnesium sulfate 0.2 g., ammonium tartrate 10 g., cane sugar (sucrose) 150 g., water to make 1 liter.

PHENOL: To liquify add 10 ml. of water to each 450 g. melted phenol. Melt by immersing bottle in hot water.

PLATING SOLUTION FOR ELECTRODES: Platinum chloride 1 - 3%, lead acetate 0.02%. Use 4 volts for about 10 minutes.

PYROGALLOL FLUID FOR ABSORBING OXYGEN: Water 500 ml., potassium hydroxide 220 g., pyrogallic acid 15 g.

POISON IVY PREVENTIVES: I. Saturated aqueous sodium thiosulfate.
II. Ferric chloride 5 g., glycerine 50 ml., water 50 ml.
III. Ferric chloride 5 g., glycerine 5 ml., 50% alcohol 100 ml.

RAFFEL'S FLUID: Water 1000 ml., potassium nitrate 0.5 g., dibasic potassium phosphate 0.06 g., magnesium sulfate 0.02 g., ferric chloride 0.001 g.

RINGERS SOLUTIONS: Cf. table in Chapter VIII

SALINE: (normal physiological) Water 1000 ml., sodium chloride 7 to 9 grams. For cold blooded animals, use 7 g. For warm blooded animals use 9 g.

SZOMBATHY'S FLUID: Gelatin 1 g., water 100 ml., sodium silicate 0.02 g., glycerine 15 ml. Dissolve the gelatin at 30°C. Cool and filter through cloth.

TOISSON'S MIXTURE: (a diluting fluid for blood) Water 160 ml., sodium sulfate 8 g., sodium chloride 1 g., glycerine 30 ml., methyl violet 0.025 g.

TYRODE'S SOLUTION: (for gut muscle, no advantage for heart muscle) Water 1000 ml., sodium chloride 8 g., potassium chloride 0.2 g., calcium chloride (anhyd) 0.2 g., magnesium chloride 0.1 g., monobasic sodium phosphate 0.05 g., sodium bicarbonate 1 g., glucose 1 g.

ULTRA-VIOLET LIGHT is removed by saturated aqueous sodium nitrate or 10% $CuSO_4 \cdot 5\ H_2O$.

VAN DER CRONE'S SOLUTION: (green organisms) Water 1 liter, potassium nitrate 1.0 g., magnesium sulfate and calcium sulfate each 0.5 g., calcium phosphate 0.25 g., ferrous phosphate 0.25 g. Make slightly acid or neutral with phosphoric acid.

VAN'T HOFF'S SOLUTION: (artificial sea water) Sodium chloride 19.0 g., magnesium sulfate 1.5 g., magnesium chloride 2.4 g., potassium chloride 0.53 g., calcium chloride (anhyd) 0.37 g., anhydrous salts dissolved and made up to 1000 ml. with glass distilled water. Also Cf. table in Chapter VIII.

CHAPTER III.

KILLING AND FIXING FLUIDS

COPPER ACETATE FORMALIN: Saturated cupric acetate in 40% formaldehyde. Dilute to about 4% for preservation of green algae.

CUPRIC-PARANITROPHENOL FLUID: (Petrunkevitch) 60% alcohol 100 ml., nitric acid 3 ml., ether 5 ml., cupric nitrate (3 H_2O) 2 g., paranitrophenol cryst. 5 g. One part formaldehyde may be added to 4-7 parts of the fluid just before use.

CUPRIC PHENOL FLUID: (Petrunkevitch) A: distilled water 100 ml., nitric acid 12 ml., cupric nitrate (3 H_2O) 8 g. B: 80% alcohol 100 ml., phenol cryst. 4 g., ether 6 ml. Use 1 part of A to 3 of B. Will not keep after mixing.

FAA: (General Biological Supply House) 50% alcohol 100 ml., 40% formaldehyde 6.5 ml., glacial acetic acid 2.5 ml.

HOLLANDE'S FLUID: Formalin sat. with picric acid 12 ml., absolute alcohol 54 ml., benzene 3 ml., and nitric acid 1 ml.

LANE'S SOLUTION: Potassium bichromate 2.5 g., mercuric chloride 5.0 g., water 100 ml.

NAVASCHIN'S FLUID: 10% chromic acid, 1.5 ml., glacial acetic acid 1 ml., formaldehyde (40%) 0.83 ml., water 32.67 ml.

PRESERVING FLUID FOR GREEN ALGAE: Potassium chrom alum 10 g., formaldehyde 5 ml., water 500 ml.

STOCKARD'S SOLUTION: Water 85 ml., formalin 5 ml., glacial acetic acid 4 ml., glycerine 6 ml.

SUSA'S FLUID: Water 80 ml., mercuric chloride 4.5 g., sodium chloride 0.5 g., trichloracetic acid 2.0 g., formalin 20 ml., glacial acetic acid 4 ml.

WORCESTER'S FLUID: 10% formalin saturated with mercuric chloride, 90 ml., glacial acetic acid, 10 ml.

OSMIUM AND PLATINUM CONTAINING FLUIDS:

Osmium and platinum fixatives are costly and often do not keep

well. Few cytologists use the same formulae, each usually wanting his favorite formula, hence it is best to keep on hand certain stock solutions, among which are small amounts of osmic acid and platinic chloride. Below are listed certain fixatives containing one or both of these reagents, also a list of stock solutions. The makeup of the fixatives from the stock solutions is given in parts by volume, and the amount desired by an investigator can be made up to the nearest multiple of the total parts indicated.

STOCK SOLUTIONS

Acetic acid, glacial
Chromic acid, 1%
Chromic acid, 1% in 1% NaCl
Formic acid
Mercuric chloride, sat. soln. in hot water
Mercuric chloride, 0.5% in 1% chromic acid
Osmic acid, 2%
Picric acid, sat. aq.soln.
Platinic chloride, 10%
Potassium dichromate, 10%

FIXATIVES:

Some of the fixatives listed here keep well and may be kept for a long time. Those which deteriorate are noted. All of these formulae are from Lee's Vade Mecum, unless otherwise stated. In making osmic acid wash off the paper covering of the glass ampoule; rinse in distilled water, and file a notch around the tube. Drop the ampoule into a clean, glass stoppered bottle of a capacity greater than the amount of osmic desired. The tube of osmic crystals may now be broken open with a heavy glass rod. As many tubes as wanted may be crushed inside the glass bottle but not over 200-300 c.c. of 2% should be kept in solution.

All osmic acid and fixatives containing it should be kept in dark bottles with well fitted glass stoppers. Osmic acid reduces slowly in the light and at high temperatures; when it is issued it should be in a brown bottle or the bottle should be covered with black paper.

When issuing fixatives the label should indicate definitely the formula used, since there are 4 Flemming's and 3 Von Rath's solutions.

KILLING AND FIXING FLUID FOR HISTOLOGICAL TECHNIQUE

Killing Fluid	Ml. Water	Ml. Glacial Acetic Acid	Ml. 100% Alcohol	g. Mercuric Chloride	g. Potassium Dichromate	Ml. Sat. Aq. Picric Acid	Ml. 10% Chromic Acid	Ml. 2% Osmic Acid	Ml. 40% Formaldehyde	g. Sodium Sulfate (anhyd)	General
Bouin		5				75			25		
B_3 (Carother's)		10				75			15		Warm to about 50°C and add 1 g. urea. Stir until dissolved.
B_{15} (Allen's)		5				75	15		25		Warm to about 50°C and add 2 g. urea. Stir until dissolved. Chromic acid should not be added until ready to use.
Carnoy 1		25	75								
Carnoy 2		10	60								Chloroform 30 Ml.
Carnoy and Lebrun		50	50	To sat.							Chloroform 50 Ml.
Champy	70				1.05		3.5	20			
Flemming (Weak)	95	0.1					2.5	5			
Flemming (Strong)	15	1					1.5	4			
Gilson	920	4	60	20							15 Ml. Conc. Nitric Acid
Hayem	200			0.5						5	1 g. Sodium Chloride
Helly	100			5	2-2.5				5	1	Add formaldehyde just before using.
Kahle's	31	1	15						6		
Kleinenberg — Mayer's Picro-sulfuric diluted with 3 times its volume of water											
Mayer (Picro-sulfuric)						100					2 Ml. Conc. H_2SO_4
Meve	15	3 drops					1.5	3-4			0.15 g. Sodium Chloride
Petrunkevitch	300	90	200	To sat.							10 Ml. Conc. Nitric Acid
O. VonRath		0.5-1		50Ml. Sat.Aq.		50		10 If desired			
Schaudinn		Trace if desired	50	100Ml. Sat.Aq.							
Zenker	100	5		5	2-2.5					1	Add acetic acid just before using.

CHAPTER IV.

STAINS AND STAINING SOLUTIONS

NOTE: Use distilled water in all solutions.

ACETO-CARMINE: (Schneider's) Mix equal volumes of glacial acetic acid and water. Saturate with powdered carmine, boil, cool and filter.

ALUM COCHINEAL: Boil powdered cochineal in 5% aqueous solution of aluminum potassium sulfate or aluminum ammonium sulfate. Filter and add a little salicylic acid to the filtrate as a preservative.

AMMONIA-CARMINE: (Ranvier) Dissolve carmine in water with a slight excess of ammonia. Evaporate to dryness, dissolve the residue in water, and filter.

AZO-CARMINE: <u>Azo-carmine GX</u> 0.2 g., boiling water 100 ml. When cool filter and add 1 ml. glacial acetic acid. The precipitate redissolves as the stain is used at 55°C. <u>Azo-carmine B</u> 0.33 g., water 100 ml., acetic acid trace.

AZAN STAIN: cf. Mallory A' and Azo-carmine.

BEST'S CARMINE: Carmine 2 g., potassium carbonate 1 g., potassium chloride 5 g., water 60 ml. Boil gently for a few minutes and cool. Then add 20 ml. of conc. ammonium hydroxide.

BORAX-CARMINE: (Grenacher's) Sodium tetraborate (borax) 4% aqueous solution 100 ml., carmine 3 g. Boil until the carmine dissolves, cool and add 100 ml. of 70% alcohol. Filter after 24 hours.

BORREL: <u>A.</u> 1% aqueous magenta (basic fuchsin) <u>B.</u> Indigo carmine 1 g., distilled water 60 ml., saturated aqueous picric acid 40 ml.

CARBOL-FUCHSIN: Fuchsin (sat. alc. sol.) 10 ml., phenol (5% aq. sol.) 90 ml.

CARBOL-FUCHSIN: (Goodpasture's) Alcohol (20%) 100 ml., phenol (melted) 1 ml., aniline 1 ml., basic fuchsin 0.5 g.

CARMALUM: (Mayer's) Carminic acid 1 g., aluminum ammonium or aluminum potassium sulfate 10 g., water 100 ml. Dissolve with heat and filter the solution when cold. Add a crystal of thymol as a preservative.

ERLICH'S TRIPLE STAIN: (triacid mixture) Orange G (sat. aq. sol.)

14 ml., acid fuchsin (sat. aq. sol.) 7 ml., water 15 ml., absolute alcohol 25 ml., methyl green (sat. aq. sol.) 12 ml., glycerin 10 ml. Each solution should be thoroughly saturated (several days). Add the ingredients in order named, shaking mixture well after each addition.

FEULGEN'S REAGENTS: cg. nucleal reaction.

GRAM'S STAIN: See Gential violet and Gram's iodine Chapter II.

HEMALUM: (Mann's) Haematein 0.5 to 2 grams, absolute alcohol 100 ml., glycerine 100 ml., water 100 ml., potassium aluminum sulfate (potassium alum) 10 g., glacial acetic acid 10 ml. Dissolve the dye in the acid, add the alcohol, glycerine and 75 ml. of the water; then dissolve the alum in the rest of the water with gentle heat and add to the other ingredients. Five grams of aluminum sulfate may be substituted for the potassium alum.

HEMALUM: (Mayer's) Hematoxylin 1 g., water 1 liter. Dissolve and add sodium iodate 0.2 g., ammonium aluminum sulfate or potassium aluminum sulfate 50 g. Heat to dissolve. When cool, filter.

HEMALUM, ACID: (Mayer's) To Mayer's hemalum add glacial acetic acid to 2%.

HEMATOXYLIN STOCK SOLUTION: May be made by dissolving 1 part of hematoxylin crystals in 10 parts of absolute alcohol. In the course of several months or a year, this solution ripens to a dark wine-red color. It may be used in making up the various hematoxylin solutions, and being ripe, will stain at once.

HEMATOXYLIN: (Delafield's) Aluminum ammonium sulfate (ammonium alum) saturated aqueous solution 100 ml. Dissolve 1 g. of hematoxylin crystals in 10 ml. of absolute alcohol, and slowly add it to the ammonium alum. Expose to air and light for several weeks to 'ripen'. Ripening may be accomplished at once with some degree of success through the addition of a few ml. of hydrogen peroxide. When ripe, filter the solution and add 25 ml. of glycerine and 25 ml. of methyl alcohol. (Cf. Hematoxylin stock solution).

HEMATOXYLIN: (Erlich's acid) Hematoxylin 2 g., absolute alcohol 100 ml., glacial acetic acid 10 ml., glycerine 100 ml., water 100 ml., aluminum ammonium sulfate or aluminum potassium sulfate 10 g. Let the mixture ripen in the light and air until it acquires a dark red color.

HEMATOXYLIN: (Galigher's) Hematoxylin crystals 0.5 g., aluminum ammonium sulfate 0.3 g., 50% alcohol 100 ml., red mercuric oxide 0.6 g. Dissolve hematoxylin and alum in the alcohol. When boiling add mercuric oxide and boil for 20 minutes in a covered vessel. Let stand overnight. Filter. Solution can be used at once.

HEMATOXYLIN: (Harris's) Dissolve 20 g. of ammonium or potassium aluminum sulfate in 200 ml. of water with the aid of heat. Dissolve 1 g. of hematoxylin crystals in 10 ml. of absolute alcohol and add to the warm alum solution. Bring rapidly to boil and add 0.5 g. of red mercuric oxide. When the solution turns dark purple, cool, and add 8 ml. of glacial acetic acid.

HEMATOXYLIN: (Heidenhain's) (iron-hematoxylin) A. Ammonium ferric sulfate (ferric alum) 2.5 g., water 100 ml. B. 10% Hematoxylin stock solution 5 ml., distilled water 100 ml.

HEMATOXYLIN: (Mann's) Make up Erlich's Hematoxylin stain with haematein instead of hematoxylin.

HEMATOXYLIN: (Mayer's) Hematoxylin stock solution 10 ml., sodium iodate 0.26 g., chloral hydrate 6.6 g., aluminum ammonium or aluminum potassium sulfate 1.2 g., water to 2 liters.

HEMATOXYLIN: (Regaud's) Hematoxylin stock solution 10 ml., glycerine 10 ml., water 80 ml.

INDIGO-CARMINE: Indigo-carmine 0.2 g., water 100 ml.

LOEFFLER'S METHYLENE BLUE: Methylene blue 0.3 g., 95% ethyl alcohol 30 ml., 0.01% potassium hydroxide 100 ml.

MALLORY'S TRIPLE STAIN: (for connective tissue) A. Acid fuchsin 0.5 g., water 100 ml. B. Anilin blue (Gruebler's water soluble) 0.5 g., Orange G (Gruebler) 2 g., 1% aqueous phosphomolybdic acid 100 ml.

MALLORY'S SOLUTION A': (also called Heidenhain's azan stain) 0.5% Azo-carmine with 5-10 drops of glacial acetic acid to each 100 ml. of solution. Do not filter. Cf. also Azo-carmine. (Courtesy of Mrs. N. Jones)

MUCI-CARMINE: (Mayer) Water 2 ml., carmine 1 g., aluminum chloride 0.5 g., 50% alcohol 100 ml. Mix in order given, heat gently until the fluid darkens (about 2 minutes); filter after 24 hours. To use, dilute with 5 to 10 volumes of water.

NUCLEAL REACTION: Feulgen's modification of the Schiff reaction for aldehydes. A. Water with an excess of sulfur dioxide. Water 200 ml., sodium bisulfite 1.3 g., 1 N hydrochloric acid 10 ml. B. Fuchsin-sulfurous acid reagent. Dissolve 1 g. of basic fuchsin in 100 ml. warm water. Filter. Add 20 ml. of 1 N hydrochloric acid and 1 g. sodium bisulfite. Let stand 24 hours. Decolorize with Norite and filter.

PARACARMINE: (Mayer's) Carminic acid 1 g., aluminum chloride 0.5 g., calcium chloride 4 g., 70% alcohol 100 ml. Dissolve and allow to settle. Filter.

PICRO-CARMINE: Conc. ammonium hydroxide 5 ml., water 50 ml., carmine 1 g. When dissolved add saturated aqueous picric acid 50 ml. Expose to air and light for 2 days, then filter.

PICRO-FUCHSIN: (Van Gieson) Acid fuchsin (1% aq. sol.) 10 ml., picric acid (sat. aq. sol.) 90 ml.

POLYCHROME METHYLENE BLUE: (for staining cell granules) Michaelis' method: Methylene blue 2 g., water 200 ml. To this solution add 10 ml. of 0.1 N sodium hydroxide. Boil for 15 minutes. After cooling add 10 ml. of 0.1 N sulfuric acid and filter.

POLYCHROME METHYLENE BLUE: Unna's method: Methylene blue 1 g., potassium carbonate 1 g., 95% alcohol 20 ml., water 100 ml. Evaporate to 100 ml. It may be used at once, or after diluting with an equal volume of anilin water. See Chapter III.

SCHARLACH R: 70% alcohol 50 ml., acetone 50 ml., Scharlach red to saturation.

THIONIN, ACID: (Frost's form) Thionin 1 g., phenol 2.5 g., glacial acetic acid 20 ml., water 400 ml.

TRIACID STAIN: See Erlich's triple stain.

UNNA-PAPPENHEIM STAIN: (modified) Pyronin Y 0.9 g., methyl green 0.1 g., 95% alcohol 9 ml., glycerine 10 ml., 0.5% phenol to 100 ml.

WEIGERT'S STAIN: (for elastic tissue) (resorcin-fuchsin) Basic fuchsin 2 g., resorcinol 4 g., water 200 ml. Heat the mixture in a porcelain dish and while boiling add 25 ml. of a 29% aqueous solution of ferric chloride ($FeCl_3 \cdot H_2O$). Stir and boil for 2-5 minutes. A precipitate forms. Filter. Discard the filtrate. Drain the filter paper dry and return the paper and precipitate to the dish. Add 200 ml. of

95% alcohol and boil, stirring constantly. Remove the paper from the solution, filter, and add alcohol to make the solution up to 200 ml. Add 4 ml. of conc. hydrochloric acid. The solution keeps well for months.

WRIGHT'S STAIN: Wright's stain (dry powder) 0.2 g., methyl alcohol (absolute, neutral, acetone free) 60 ml. Filter after standing for 24 hours.

STAIN SOLUBILITIES

References: Conn, Biological Stains, 1946; Holmes, Stain Technology, 1929

Dye solubilities at 26°C listed as grams of anhydrous dye per 100 ml. of saturated solution selected from the above references and printed with the permission of Dr. H. J. Conn.

Aniline dyes are commonly used as saturated solutions, aqueous, or alcoholic, unless other concentrations are given.

KEY TO BIOLOGICAL USE OF THE STAINS:

B - bulk
C - cytoplasmic
F - fat
I - indicator
N - nuclear
P - perfusion
V - vital

Synonyms of the dyes are given in parentheses.

Color Index Number	Name of Dye	Water	95% Alcohol	Strength solution used	Use
1027	Alizarin	Nil	0.125		I,C,V.
1034	Alizarin red S (Alizarin red, water soluble; Alizarin carmine)	7.69	0.15		N,V.
40	Alizarol orange G (Alizarin yellow R; Mordant yellow PN; Orange R; Anthracene yellow RN; Alizarin orange)	0.40	0.57		
36	Alizarin yellow GW (Alizarin yellow GG; Anthracene yellow GG; Mordant yellow 2GT)	25.84	0.04		
184	Amaranth (Naphthol red S,C or O; Fast red; Bordeaux; Bordeaux SF; Victoria rubin O; Azo rubin; Wool red)	7.20	0.01		C.
847	Amethyst violet (Heliotrope B; Iris violet)	3.12	3.66		N.

Color Index Number	Name of Dye	Water	95 % Alcohol	Strength Solution Used	Use
655	Auramin O (Canary yellow; Pyoktaninum aureum; Pyoktanin yellow)	0.74	4.49		V,N.
12	Aurantia (Imperial yellow)	Nil	0.33		C.
724	Aurin (Rosolic Acid)	0.12	40.0		I.
146	Azo acid yellow	2.17	0.81		
448	Benzopurpurin 4B (Cotton red 4B; Dianil red 4B; Diamin red 4B; Sultan 4B; Direct red 4B)	---	0.13		C,V.
280	Biebrich scarlet (Croceine scarlet 5R; Ponceau B; Double scarletBSF; Scarlet B, or EC)	---	0.05		C.
332	Bismarck brown R (Bismarck brown GOOO; Brown R,AT,C or N; Manchester brown EE; Vesuvin NR,B, R; Basic brown BR or BXN)	1.10	0.98		N.
331	Bismarck brown Y (Vesuvin; Phenylene brown; Manchester brown; Excelsior brown; Basic brown G, GX, or GXP)	1.36	1.08		V,C,B.
88	Bordeaux red (Fast red B, BN or P; Cerasin R; Archelline 2B; Azo-Bordeaux; Acid Bordeaux)	3.83	0.19	1% aq.	C.
252	Brilliant crocein	5.04	0.06		
1239	Carmine	0.3	----		N,B.
29	Chromotrope 2R (Chromotrope N2R; Chromotrope blue 2R; XL Carmoisine 6R; Fast fuchsin G; Acid phloxine GR)	19.30	0.17		P,C.
21	Chrysoidin R (Cotton orange; Cerotin orange)	0.23	0.99		N.
20	Chrysoidin Y (Brown salt R; Dark brown salt R)	0.86	2.21		N,B,V.
370	Congo red (Congo; Cotton red B or C; Direct red C, R or Y)	----	0.19		C,B,I.
---	Cresyl violet (Cresylecht violet or cresyl fast violet)(Nat.An.Co.)	0.38	0.25		N,C,B.
89	Crystal ponceau 6R (Ponceau 6R)	0.80	0.06		F.
681	Crystal violet (chloride) (Violet C,G or 7B; Hexamethyl violet; Methyl violet 10B; Gentian violet)	1.68	13.87	1% aq.	N,V.
	Crystal violet (iodide)	0.035	1.78		N,V.
	Crystal violet (chloride) resorcin ad. prod.	0.28	13.84		N,V.
	Crystal violet (chloride) hydroquin. ad. prod.	0.30	8.39		N,V.
	Crystal violet (chloride) pyrocatechin ad. prod.	0.79	24.87		N,V.
715	Cyanol extra	1.38	0.44		P.

Color Index Number	Name of Dye	Water	95 % Alcohol	Strength Solution Used	Use
771	Eosin B (Na salt) (Eosin BN, BA, BW, DHV; Saffrosin; Eosin scarlet; Scarlet J, JJ, V; Nopalin G; Imperial red; Eosin scarlet B)	39.11	0.75		C.
768	Eosin Y (Na salt) (Eosin, water soluble; Bromo acid J, TS, XL or XX; Bromo fluorocein; Bronze bromo ES)	44.20	2.18	0.5% aq.	C.
	Eosin Y (Mg salt)	1.43	0.28		C.
	Eosin Y (Ca salt)	0.24	0.09		C.
	Eosin Y (Ba salt)	0.18	0.06		C.
130	Erika B	0.64	0.17		
254	Erythrin X	6.41	0.06		
773	Erythrosin (Mg salt)	0.38	0.52		C.
	Erythrosin (Ca salt)	0.15	0.35		C.
	Erythrosin (Ba salt)	0.17	0.04		C.
	Erythrosin, bluish (Na salt) (Erythrosin B, N, or JN; Pyrosin B; Eosin J; Iodeosin; Dianthine B)	11.10	1.87	1% aq.	C, I.
770	Ethyl eosin (Eosin, alcohol soluble; Eosin S)	0.03	1.13	0.5% al.	C.
---	Fast green FCF	16.04	0.35		C.
176	Fast red A (Fast red AV, AL•BX, S or O; Cerasin; Rubidin; Cardinal Red; Roccellin)	1.67	0.42		
16	Fast yellow (Acid yellow; Fast yellow FY, G, S, BG;	18.40	0.24		C.
766	Fluorescein (color acid)	0.03	2.21		C.
	Fluorescein (Na salt)(Uranin)	50.20	7.19		C.
	Fluorescein (Mg salt)	4.51	0.35		C.
	Fluorescein (Ca salt)	1.13	0.41		C.
	Fluorescein (Ba salt)	6.54	0.56		C.
677	Fuchsin, basic (Fuchsin RFN; Magenta; Basic rubin; Anilin)	0.30	10.00		N.
676	Pararosanilin (chloride) (Basic rubin; Parafuchsin; Paramagenta)	0.26	5.93		N.
---	Pararosanilin (acetate)	4.15	13.63		N.
---	Rosanilin (chloride)(Magenta I)	0.93	8.16		N.
692	Fuchsin, acid (Fuchsin S, SN, SS, ST, or S III; Acid magenta; Acid rubin)	12.00	0.3		C.
678	New fuchsin (chloride)(Isorubin; Fuchsin NB; Magenta III)	1.13	3.20		N.
	Gentian violet (Methyl violet 2B)	1.50	3.00	1% al.	N.
666	Guinea green B	28.40	7.30		
1180	Indigo carmine (Indigotine Ia)	1.68	0.01		C.
133	Janus green B (Diazin green S; Union green B)	5.18	1.12	0.1-1% aq.	V, N, B.

Color Index Number	Name of Dye	Water	95 % Alcohol	Strength Solution Used	Use
670	Light green SF yellowish (Light green 2G,S,2GN; Fast acid green N; Acid green)	20.35	0.82	0.5% al.	C.
657	Malachite green (oxalate) (Victoria green; New Victoria green extra,O,I, or II;Diamond green B,BX, or P extra; Solid green O; Light green N)	7.60	7.52		C.
9	Martius yellow, Na salt (Naphthol yellow; Manchester yellow)	4.57	0.16		C.
	Martius yellow, Ca salt	0.05	1.90		C.
138	Metanil yellow (Orange MNO or MN; Acid yellow R; Soluble yellow OL; Yellow M; Tropaeolin G)	5.36	1.45		C.
684	Methyl green (Double green SF; Light green)	7.00	0.25	1% aq.	N.
142	Methyl orange (Orange III; Helianthin; Gold orange MP; Tropaeolin D)	0.52	0.08		I,C.
	Methyl orange (acid)	0.015	0.015		I,C.
680	Methyl violet (Gentian violet) (Dahlia B; Paris violet; Pyoktaninum coeraleum)	2.93	15.21	0.5-2% aq.	N,V.
922	Methylene blue (chloride) (Swiss blue	3.55	1.48		N,V,I.
	Methylene blue ($ZnCl_2$ double salt)	2.75	0.05		N,V,I.
	Methylene blue (iodide)	0.09	0.13		N,V,I.
924	Methylene green	1.46	0.12		N,V.
10	Naphthol yellow G	8.96	0.025		C.
152	Narcein	10.02	0.06		C.
825	Neutral red (chloride) (Toluylene red)	5.64	2.45	1% aq.	V,N,C,I.
	Neutral red (iodide)	0.15	0.16		V,N,C,I.
826	Neutral violet	3.27	2.22		I,N.
927	New methylene blue N (Methylene blue NN)	13.32	1.65		N.
728	New Victoria blue R (New Victoria blue B or R; Corn blue B	0.54	3.98		V,N.
520	Niagara blue 4B (Pontamine sky blue 5BX; Direct sky blue; Benzo sky blue)	13.51	Nil		F.
914	Nile blue 2B	0.16	0.62		
73	Oil red O (Confused with SudanII)	Nil	0.39		F.
150	Orange I (Naphthol orange; Tropaeolin G,000 No.1)	5.17	0.64		C,I.

Color Index Number	Name of Dye	Water	95 % Alcohol	Strength Solution Used	Use
151	Orange II (Gold orange; Orange A,P,R; Acid orange II,Y or A; Orange extra; Mandarin G; Tropaeolin 000 No.2)	11.37	0.15		C.
27	Orange G (Wool orange 2G; Crystal orange GG)	10.86	0.22		C.
143	Orange IV (Orange N; Acid yellow D; Tropaeolin 00)	0.16	0.20		I.
676	Pararosanilin (chloride) (Magenta O; Basic rubin; Parafuchsin; Paramagenta)	0.26	5.93		N.
---	Pararosanilin (acetate)	4.15	13.63		N.
714	Patent blue A	8.40	5.23		P.
	Phenolphthalein	0.04	10.00		I.
774	Phloxine (Na salt) (Erythrosin BB or B extra; New pink)	50.90	9.02		C.
	Phloxine (Mg salt)	20.84	29.10		C.
	Phloxine (Ca salt)	3.57	0.45		C.
	Phloxine (Ba salt)	6.01	1.17		C.
7	Picric acid	1.18	8.96		C.
28	Ponceau 2G	1.75	0.21		P.
186	Ponceau 6R	12.98	0.01		P.
	Purpurine 4B		0.13		N.
741	Pyronin B (iodide)	0.07	1.08		N.
739	Pyronin Y (Pyronin G)	8.96	0.60		N.
148	Resorcin yellow	0.37	0.19		C.
749	Rhodamine B (Rhodamine O; Brilliant pink B)	0.78	1.47		V,C.
750	Rhodamine G	1.34	6.31		C.
176	Roccellin (Fast red A,AV,AL,BX,S or O; Cerasin; Rubidin; Cardinal red)	1.67	0.42		C.
	Rosanilin (Chloride (Magenta I)	0.39	8.16		N.
779	Rose bengal (Na salt)	36.25	7.53		C.
	Rose bengal (Mg salt)	0.48	1.59		C.
	Rose bengal (Ca salt)	0.20	0.07		C.
	Rose bengal (Ba salt)	0.17	0.05		C.
841	Safranin O (Gossypimine; Cotton red; Safranin Y or A)	5.45	3.41	1% aq.	N,V.
689	Spirit blue (Anilin blue, alcohol soluble; Gentiana blue 6B; Light blue; Lyon blue; Paris blue)	Nil	1.10	0.3% aq.	C,B.

Color Index Number	Name of Dye	Water	95 % Alcohol	Strength Solution Used	Use
24	Sudan I	Nil	0.37		F.
73	Sudan II (Oil scarlet; Fast oil orange II; Red B; Fat ponceau; Orange RR)	Nil	0.39		F.
248	Sudan III (Sudan G; Tony red; Scarlet B, fat soluble; Fat ponceau G; Oil red AS,O,B or 3B; Cerasin red)	Nil	0.15		F.
258	Sudan IV (Scarlet red; Fat ponceau; Fat ponceau R or LB; Cerotine ponceau 3B; Oil red IV)	Nil	0.09		F.
920	Thionin (Lauth's violet)	0.25	0.25		N,V.
925	Toluidine blue O (Methylene blue T50 or T extra)	3.82	0.57	0.3-1% aq.	N,V.
728	Victoria blue R (New Victoria blue B or R; Corn blue B)	0.54	3.98		V,N.
690	Victoria blue 4R (Fat blue 4R)	3.23	20.49		N.
569	Victoria green 3B	0.04	2.24		
8	Victoria yellow	1.66	1.18		C.

CHAPTER V.

STANDARD CHEMICAL SOLUTIONS, STOCK SOLUTIONS AND SOLUBILITIES

Preparation of Standard Solutions:

Reagent grade chemicals and freshly distilled water should be used for all solutions. Weighings should be carried out in the low humidity room, using calibrated weights. Calibrated glassware should be used in all volumetric operations. Standard solutions should be issued in clean, dry Pyrex bottles. Rubber stoppers (cleaned in hot sodium hydroxide solution, then washed and dried) should be used for alkali solutions; glass stoppered Pyrex bottles should be used for the other solutions.

Standard solutions should be issued only by the member of the staff assigned to this work or by the person in charge. The accuracy of the solution should be indicated on the label of each container. When an accurately standardized solution is requested by an investigator, the staff member should find out whether extreme accuracy is needed. If not, an attempt should be made to convince the investigator that an approximate solution is adequate.

A. Standard Acids and Bases:

The base solutions are standardized with solid potassium acid phthalate while the acid solutions are standardized with the standard bases. Thus, potassium acid phthalate is the reference standard for all acid and base solutions and the best grade of this compound should be used.

The potassium acid phthalate is dried at 110°-115° for two or three hours and then stored in a desiccator in the low humidity room. Samples are weighed in the Erlenmeyer flasks to be used in the titrations, and the weighings are carried out in the low humidity room.

Normal Sodium Hydroxide:

Saturated sodium hydroxide solution should be prepared for use during the following year and stored in a paraffined bottle closed by a cleaned rubber stopper. The sodium carbonate precipitates, leaving a clear supernatant solution which should be pipetted or carefully decanted from the carbonate.

For each liter of normal sodium hydroxide, dilute 60 ml. of the saturated sodium hydroxide solution to one liter with freshly boiled distilled water and store in a paraffined bottle protected with a soda-lime tube. Adjust as closely as possible to one normal by single runs against potassium acid phthalate. Finally, accurately standardize the

solution by at least six runs against 7-8 gram samples of potassium acid phthalate, titrating to a faint pink with phenolphthalein.

Tenth Normal Sodium Hydroxide:

The procedure is the same as for normal sodium hydroxide, except that 6 ml. of the saturated sodium hydroxide solution is used per liter of solution, and 0.8-0.9 gram samples of potassium acid phthalate are used.

Normal Hydrochloric Acid:

For each liter of normal hydrochloric acid, dilute 86 ml. of the concentrated acid, sp. gr. 1.18, to one liter with distilled water. Adjust as closely as possible to one normal by titration with the standardized one normal sodium hydroxide. Finally, accurately standardize the acid by at least six titrations with 40-45 ml. samples of the standard base, to a faint pink with phenolphthalein.

Tenth Normal Hydrochloric Acid:

The procedure is the same as for normal hydrochloric acid, except that 8.6 ml. of concentrated acid, sp. gr. 1.18, is used per liter of solution, and the standard tenth normal sodium hydroxide is used in the titrations.

Normal Sulfuric Acid:

For each liter of normal sulfuric acid, slowly add 27 ml. of concentrated sulfuric acid, sp. gr. 1.84, to about 250 ml. of distilled water, allow to cool, and dilute to one liter. Proceed as with normal hydrochloric acid.

Tenth Normal Sulfuric Acid:

Proceed as for normal sulfuric acid, using 2.7 ml. of concentrated sulfuric acid, sp. gr. 1.84, per liter of solution. Standardize with tenth normal sodium hydroxide.

Normal Acetic Acid:

For each liter of normal acetic acid, dilute 60 ml. of glacial acetic acid to one liter with distilled water. Proceed as with normal hydrochloric acid.

B. Other Standard Solutions:

Tenth Normal Potassium Iodate:

Weigh out 3.5672 grams of dried potassium iodate and make up to one liter with distilled water. This solution keeps very well and need not be freshly prepared each year.

Tenth Normal Sodium Thiosulfate:

For each liter of solution, dissolve 24.8 grams of hydrated sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$, in freshly boiled distilled water, add 10 ml. of tenth normal sodium hydroxide, and dilute to one liter. To standardize

against potassium iodate, dissolve 1-2 grams of reagent grade potassium iodide in 200 ml. of water in a 500 ml. Erlenmeyer flask, add ten drops of concentrated hydrochloric acid and test for free iodine with starch. If no free iodine is present, add 25.00 ml. of the potassium iodate solution and titrate the liberated iodine with the thiosulfate immediately. The thiosulfate is added until the solution is a pale yellow, then 2-3 ml. of starch solution are added and the titration continued until the solution is colorless. The average of several titrations which agree closely should be taken as the normality of the thiosulfate solution.

The thiosulfate solution should be prepared a year in advance. After this time its concentration changes very little.

Tenth Normal Potassium Permanganate:

Dissolve 3.25 grams of potassium permanganate in one liter of distilled water and boil the solution for 10-15 minutes, let stand overnight and then filter through sintered glass or asbestos. Standardize with tenth normal thiosulfate by measuring out 25.00 ml. of the permanganate solution into a 500 ml. Erlenmeyer flask containing 3 grams of potassium iodide and 5 ml. of concentrated hydrochloric acid dissolved in 50 ml. of water. Let stand in the dark for 5 minutes, dilute to about 200 ml. with water and titrate with the thiosulfate, using starch as the indicator.

The addition of 10 grams of potassium hydroxide per liter of the permanganate solution increases its stability.

Tenth Normal Potassium Bichromate:

Dissolve 4.90 grams of potassium bichromate in distilled water and make up to one liter. Standardize with tenth normal thiosulfate by measuring out 25.00 ml. of the bichromate solution into a 750 ml. Erlenmeyer flask containing 10 ml. of concentrated hydrochloric acid and 3 grams of potassium iodide in 50 ml. of water. Let stand in the dark for 5 minutes, dilute to about 400 ml. with water and titrate with the thiosulfate, using starch as the indicator.

Owing to the green color of the final solution, it is advisable to carry out the titration under artificial light.

Normal Silver Nitrate:

Weigh out 169.89 grams of silver nitrate, previously dried at 110°, dissolve in distilled water, make up to one liter and protect the solution from the light. This solution is usually adequate for the standardization of chloride solutions but it may be standardized by gravimetric determination as silver chloride. When greater accuracy is needed, the dry crys-

tals may be fused for 15 minutes at 220°-250°.

C. Approximate Standards:

Prepare at the beginning of the season about 14 liters of 1.0 N hydrochloric acid by diluting the acid according to the information in the table on the strengths of acids; and of 1.0 N sodium hydroxide by weighing out the proper amount of alkali and making up to volume. Other approximate standards are to be made as required and every effort should be made to issue the proper solution thereby saving the stocks of the specially accurate solutions when these are not required.

STRENGTH OF STOCK ACIDS AND BASES

Substance	Molecular Weight	Molarity	Normality	Specific Gravity	Percent	Grams/ Liter	ml. to make one l. of 1N
Acids							
Acetic	60.05	17.36	17.36	1.05	99.8	1048	57
Butyric	88.06	10.78	10.78	0.959	98-100	949	93
HCl	36.47	11.50	11.50	1.19	37	440	83
Lactic	90.08	11.25	11.25	1.21	85-90	1030	87
Nitric	63.02	15.82	15.82	1.42	70.5	1001	63
Phosphoric	98.00	14.75	44.25(3H)	1.70	85	1445	23 (3H)
Sulfuric	98.08	18.01	36.03	1.84	94	1730	28
Bases							
Ammonium Hydroxide	17.03	14.7	14.7	0.90	28(NH_3)	252	67

SOLUBILITY OF COMPOUNDS*

Solubility is expressed in grams of solute per 100 ml. of solvent at 20°C.

KEY: s - soluble; sl.s - slightly soluble; inf - infinitely soluble; i - insoluble; vs - very soluble

Substance	Formula	Mol. Wt.	Sol. H_2O	Sol. Alcohol
Acid				
Boric	H_3BO_3	61.84	5.15	5.56
Citric	$H_3C_6H_5O_7 \cdot H_2O$	210.11	135	116
Oxalic	$(COOH)_2 \cdot 2H_2O$	126.06	10	23.7
Phosphomolybdic	$20MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$	3939.78	0.4	vs
Picric	$C_6H_2(OH)_2(NO_2)_3$	229.08	1.0	4.91
Tartaric	$C_2H_2(OH)_2(COOH)_2$	150.07	139	19.85
Trichloracetic	CCl_3COOH	163.40	120	s
Aluminum Chloride	$AlCl_3$	133.34	69.87	100

Substance	Formula	Mol. Wt.	Sol. H_2O	Sol. Alcohol
Alum				
Amm. Iron	$Fe_2(SO_4)_3\ (NH_4)_2SO_4 \cdot 24\ H_2O$	964.40	124	i
Potas. Chrom.	$Cr_2(SO_4)_3K_2SO_4 \cdot 24\ H_2O$	1006.51	20	i
Potas. Alumin.	$Al_2(SO_4)_3K_2SO_4 \cdot 24\ H_2O$	948.77	11.4	i
Amidol	diaminophenol HCl	197.01	20.5	sl.s
Ammonium				
Acetate	$NH_4C_2H_3O_2$	77.06	148	v.s.
Chloride	NH_4Cl	53.50	38	0.6
Molybdate	$(NH_4)_2MoO_4$	196.03	3.5	i
Nitrate	NH_4NO_3	80.05	120	3.8
Oxalate	$(NH_4)_2C_2O_4 \cdot H_2O$	142.12	4	i
Sulfate	$(NH_4)_2SO_4$	132.14	75	i
Barium				
Chloride	$BaCl_2 \cdot 2H_2O$	244.32	35.7	i
Hydroxide	$Ba(OH)_2 \cdot 8H_2O$	315.51	3.5	sl.s
Calcium				
Chloride	$CaCl_2$	110.98	74.5	s
Chloride	$CaCl_2 \cdot 2H_2O$	147.03	100	s
Chloride	$CaCl_2 \cdot 6H_2O$	219.09	300	s
Carbon Tet.	CCl_4	153.84	0.08	inf.
Chloroform	$CHCl_3$	119.39	1.0	inf.
Chromium Oxide	CrO_3	100.01	170	s
Copper				
Acetate (ic)	$Cu(C_2H_3O_2)_2 \cdot H_2O$	199.63	7.2	7.14
Chloride (ic)	$CuCl_2 \cdot 2H_2O$	170.52	126	s
Chloride (ous)	Cu_2Cl_2	198.05	0.0062	i
Sulfate (ic)	$CuSO_4$	159.63	40	i
Sulfate (ic)	$CuSO_4 \cdot 5H_2O$	249.71	60	i
Dextrose (glucose)	$C_6H_{12}O_6 \cdot H_2O$	198.14	82	2
Ether	$C_2H_5OC_2H_5$	74.10	7.5	inf.
Glycine	CH_2NH_2COOH	75.04	25	0.043
Glycylglycine	$NH_2CH_2CONHCH_2COOH$	132.12	19.8	sl.s
Hydroquinone	$C_6H_4(OH)_2$	110.08	6.1	v.s.
Iodine	I_2	253.84	0.029	20.5
Iron				
Chloride (ic)	$FeCl_3 \cdot 6H_2O$	270.31	300	s
Sulfate (ous)	$FeSO_4 \cdot 7H_2O$	278.02	28	i
Lactose	$C_{12}H_{22}O_{11} \cdot H_2O$	360.31	17	i
Lead				

Substance	Formula	Mol. Wt.	Sol. H_2O	Sol. Alcohol
Acetate	$Pb(C_2H_3O_2)_2 \cdot 3H_2O$	379.30	50	i
Chloride	$PbCl_2$	278.11	1.0	i
Lithium				
Carbonate	Li_2CO_3	73.88	1.33	i
Chloride	$LiCl$	42.40	75	2.48
Magnesium				
Chloride	$MgCl_2 \cdot 6H_2O$	203.33	167	50
Sulfate	$MgSO_4 \cdot 7H_2O$	246.50	71	s
Maltose	$C_{12}H_{22}O_{11} \cdot H_2O$	360.31	100	v.sl.s.
Manganese Chloride	$MnCl_2 \cdot 4H_2O$	197.91	225	s
Mercuric Chloride	$HgCl_2$	271.52	6.9	33
Osmium Tetroxide[1]	OsO_4	254.80	6	s
Potassium				
Acetate	$KC_2H_3O_2$	98.12	253	33
Bromide	KBr	119.01	63	0.5
Carbonate	K_2CO_3	138.19	112	i
Bicarbonate	$KHCO_3$	100.10	27	i
Chloride	KCl	74.55	34.7	i
Cyanide	KCN	65.10	50	s
Dichromate	$K_2Cr_2O_7$	294.21	14	i
Ferricyanide	$K_3Fe(CN)_6$	329.18	40	i
Ferrocyanide	$K_4Fe(CN)_6 \cdot 3H_2O$	422.32	30	i
Hydroxide	KOH	56.10	110	v.s.
Iodide	KI	166.03	140	14.3
Nitrate	KNO_3	101.10	31.6	i
Oxalate	$K_2C_2O_4 \cdot H_2O$	184.21	33	i
Permanganate	$KMnO_4$	158.03	10	i
Phosphate (mono)	KH_2PO_4	136.14	33	i
Phosphate (di)	K_2HPO_4	174.22	v.s.	v.s.
Acid Phthalate	$KHC_8H_4O_4$	204.22	10	i
Sulfate	K_2SO_4	174.26	11.7	i
Thiocyanate	$KCNS$	97.17	185	s
Silver Nitrate	$AgNO_3$	169.89	288	v.sl.s.
Sodium				
Acetate	$NaC_2H_3O_2 \cdot 3H_2O$	136.06	100	2.18
Borate, tetra	$Na_2B_4O_7 \cdot 10H_2O$	381.43	7	v.sl.s.
Carbonate	Na_2CO_3	105.99	24	sl.s.

Substance	Formula	Mol. Wt.	Sol. H_2O	Sol. Alcohol
Carbonate	$Na_2CO_3 \cdot 10H_2O$	286.15	65	i
Bicarbonate	$NaHCO_3$	84.01	10	sl.s.
Chloride	$NaCl$	58.46	36.5	sl.s.
Chromate	$Na_2CrO_4 \cdot 10H_2O$	342.16	60	sl.s.
Citrate	$Na_3C_6H_5O_7 \cdot 2H_2O$	294.10	50	sl.s.
Cyanide	$NaCN$	49.01	50	sl.s.
Hydroxide	$NaOH$	40.01	103	v.s.
Nitrate	$NaNO_3$	85.01	93	sl.s.
Oxalate	$Na_2C_2O_4$	134.01	3.7	i
Phosphate (mono)	$NaH_2PO_4 \cdot H_2O$	138	110	i
Phosphate (di)	$Na_2HPO_4 \cdot 12H_2O$	358.24	17	i
Phosphate (di)	$Na_2HPO_4 \cdot 2H_2O$	178.05	50	i
K Tartrate	$NaKC_4H_4O_6 \cdot 4H_2O$	282.19	60	v.sl.s.
Sulfate	Na_2SO_4	142.06	14	i
Sulfate	$Na_2SO_4 \cdot 10H_2O$	322.22	35	i
Sulfite	Na_2SO_3	126.06	26	sl.s.
Bisulfite	$NaHSO_3$	104.07	25	i
Thiocyanate	$NaCNS$	81.07	139	v.s.
Thiosulfate	$Na_2S_2O_3 \cdot 5H_2O$	248.20	250	i
Veronal	$NaC_8H_{11}N_2O_3$	206.18	20	sl.s.
Sucrose	$C_{12}H_{22}O_{11}$	342.30	200	0.9
Thymol	$C_6H_3(CH_3)(OH)(C_6H_7)$	150.21	0.09	350
Urea	$CO(NH_2)_2$	60.05	100	15.8
Uranium Nitrate	$UO_2(NO_3)_2 \cdot 6H_2O$	502.18	200	v.s.
Urethane				
Ethyl	$NH_2COOC_2H_5$	89.08	100	166
Phenyl	$C_6H_5NHCOOC_2H_5$	165.19	sl.s.	v.s.
Zinc				
Chloride	$ZnCl_2$	136.29	430	100
Sulfate	$ZnSO_4 \cdot 7H_2O$	287.56	96	sl.s.

*For additional information cf. Handbook of Chemistry and Physics; Chemical Dictionary; Merck's Index; Seidell's Solubilities of Inorganic and Organic Substances.

1. Forms the acid in water.

GRAVIMETRIC FACTORS

A gravimetric factor is a stoichiometric ratio between the weights of two substances. In the case of Table A, it is the ratio of the molecular weights of the hydrated/anhydrous salts. To obtain the weight of hydrate equivalent to a given amount of anhydrous salt, multiply the weight of anhydrous salt by the factor. In the case of Table B, the factor is the ratio of equivalent weights as shown in the formula fraction. To obtain the weight of substance in the numerator equivalent to a known weight of substance in the denominator, multiply the latter weight by the factor.

TABLE A: Hydrated:Anhydrous Salts

Cation	Formula of Hydrate	Factor
Acids	$H_3Citric+H_2O$	1.095
	$H_2C_2O_4+2H_2O$	1.401
Ammonium	$(NH_4)_2C_2O_4+H_2O$	1.145
Calcium	$CaCl_2+6H_2O$	1.974
	$CaCl_2+2H_2O$	1.325
	$Ca(NO_3)_2+4H_2O$	1.440
	$CaC_2O_4+H_2O$	1.141
	$CaSO_4+2H_2O$	1.264
Copper	$CuCl_2+2H_2O$	1.268
	$Cu(NO_3)_2+3H_2O$	1.287
	$CuSO_4+5H_2O$	1.564
Dextrose	$C_6H_{12}O_6+H_2O$	1.100
Iron	$FeCl_3+6H_2O$	1.667
	$FeCl_2+4H_2O$	1.568
Lead	$Pb(OAc)_2+3H_2O$	1.165
Lithium	$Li_2SO_4+H_2O$	1.164
Magnesium	$MgBr_2+6H_2O$	1.587
	$MgCl_2+6H_2O$	2.135
	$MgSO_4+7H_2O$	2.048
Manganese	$MnCl_2+4H_2O$	1.572
	$MnSO_4+4H_2O$	1.477
	$MnSO_4+H_2O$	1.119
Potassium	$K_4Fe(CN)_6+3H_2O$	1.147
Sodium	$NaOAc+3H_2O$	1.659
	$Na_2CO_3+H_2O$	1.170
	$Na_2CrO_4+4H_2O$	1.486
	$Na_3Citric+2H_2O$	1.140
	$Na_2MoO_4+2H_2O$	1.176
	$NaH_2PO_4+H_2O$	1.150

Cation	Formula of Hydrate	Factor
	$Na_2HPO_4 \cdot 12H_2O$	2.522
	$Na_3PO_4 \cdot 12H_2O$	2.319
	$Na_2SO_4 \cdot 10H_2O$	2.269
	$Na_2S \cdot 9H_2O$	3.078
	$Na_2SO_3 \cdot 7H_2O$	2.001
	$Na_2S_2O_3 \cdot 5H_2O$	1.560
Strontium	$SrCl_2 \cdot 6H_2O$	1.682
Zinc	$ZnSO_4 \cdot 7H_2O$	1.781

TABLE B: SUBSTITUTION OF IONS

Ions Exchanged	Formula Fraction	Factor
Sodium→Potassium	$KHCO_3/NaHCO_3$	1.192
	K_2HPO_4/Na_2HPO_4	1.227
	$NaH_2PO_4 \cdot H_2O/KH_2PO_4$	1.014
Strontium→Calcium	$CaCl_2 \cdot 2H_2O/SrCl_2$	0.927
Chloride→Bromide	$NaBr/NaCl$	1.761
	KBr/KCl	1.576
	$CaBr_2 \cdot 2H_2O/CaCl_2 \cdot 2H_2O$	1.604
	$MgBr_2 \cdot 6H_2O/MgCl_2 \cdot 6H_2O$	1.437
Chloride→Iodide	$NaI/NaCl$	2.564
	KI/KCl	2.228
	$CaI_2 \cdot 6H_2O/CaCl_2 \cdot 2H_2O$	2.732
	$MgI_2 \cdot 8H_2O/MgCl_2 \cdot 6H_2O$	2.076
Chloride→Nitrate	$NaNO_3/NaCl$	1.454
	KNO_3/KCl	1.356
	$Ca(NO_3)_2 \cdot 4H_2O/CaCl_2 \cdot 2H_2O$	1.605
	$Mg(NO_3)_2 \cdot 6H_2O/MgCl_2 \cdot 6H_2O$	1.261

CHAPTER VI.

INDICATORS AND INDICATOR SOLUTIONS

Revised 1951 by J. D. Ostrow

Reference: Clark, W. M., The Determination of Hydrogen Ions. (3rd Ed)

INDICATOR SOLUTIONS:

In the colorimetric determination of pH's, aqueous solutions containing 0.04% of the indicator are generally used. However, to simplify preparation and increase stability, stock solutions of 0.4% concentration are first prepared, and the test solutions made up as needed by dilutions of the stock. Since the indicators, as such, are insoluble in water, they are first converted to their monosodium salts, prior to dissolution in the specified volume of water.

To prepare the 0.4% stock solutions: weigh exactly 1.00 g. of the indicator into an agate mortar. Add the number of ml. of 0.05N NaOH specified in the table below, and grind with an agate pestle until dissolved. Transfer quantitatively, with rinsing, to a 250 ml. volumetric flask, and dilute to the mark with distilled water.

To prepare the 0.04% test solutions: dilute 1 part of the stock solution with 9 parts of distilled water, using accurately calibrated pipettes and flasks.

INDICATOR SETS:

For determination of pH's to the nearest 0.1 pH unit, sets of indicator color standards are used. Each set covers the detectable range of color change of a given indicator, as listed in the table below, and proceeds in steps of 0.2 pH units, intermediate values being estimated visually.

Each set consists of nine colorimetrically matched 15 X 150 mm. test tubes, each calibrated at 10 ml. If the tubes cannot be matched on a colorimeter, the following method will suffice: all tubes whose 10 ml. graduation falls at the same height above the base of the tube will have the same inside diameter, and therefore be colorimetrically identical, except for variations in the thickness of the walls of the tubes. They may, therefore, be used in the same indicator set, with little resultant error.

Each tube is filled to the mark with buffer solution at the proper pH, the bottom of the meniscus being read in each case. Five drops, or 0.25 ml., of the 0.04% indicator test solution is then added. The tube

is stoppered with a cork which is protected from the solution by cellophane, and then inverted several times until thoroughly mixed. It is then labelled with the name of the indicator, the volume, and the concentration of the indicator solution used, and the pH of the buffer. Tubes must be freshly prepared each summer, after thorough washing, though labels may be reused without removal if they have been protected by Scotch Tape or label varnish. Stability is also enhanced by storage in the dark, but in any case, the sets are good only for three months at the most.

A set is issued in a test tube rack with an empty, matched tube and a dropping bottle of the indicator solution. To avoid errors in the volume of indicator used, the same bottle should be used in making the standard tubes, since this will insure that the drops are all from the same dropper and, therefore, of identical size.

To use the sets, the proper indicator is first determined by adding a drop of various indicators to small amounts of the unknown. The empty tube of the proper set is then filled to the mark with the unknown solution, and five drops of the indicator solution added. After thorough mixing, the color of the unknown tube is matched with those of the standards, using a comparator block. Note that the most commonly used indicator is Phenol Red, since it exhibits its maximum color gradation in the range of physiological pH's.

TABLE OF INDICATORS

In each case, add 0.25 ml. (5 drops) of 0.04% indicator solution to 10 ml. of the solution to be tested.

Indicator	pH Range	ml. of 0.05N NaOH per gram dye	Color Change
*Thymol Blue	1.2-2.8	43	Red — Yellow
Brom Phenol Blue	3.0-4.6	30	Yellow — Blue Violet
Brom Cresol Green	3.8-5.4	29	Yellow — Blue
Chlor Phenol Red	4.8-6.4	47	Yellow — Red
Brom Cresol Purple	5.2-6.8	37	Yellow — Violet
Brom Thymol Blue	6.0-7.6	32	Yellow — Blue
Phenol Red	6.8-8.4	57	Yellow — Red
Cresol Red	7.2-8.8	53	Yellow — Red
Meta Cresol Purple	7.4-9.0	53	Yellow — Purple
Thymol Blue	8.0-9.6	43	Yellow — Blue

*Thymol Blue has two ranges, one acid and one alkaline. In the acid range, use twice as much indicator as usual, that is, 10 drops (0.5 ml.) of 0.04% solution per 10 ml. of unknown.

CHAPTER VII.

BUFFERS

Each table here given is for preparing a <u>given volume</u> of buffer, containing a specified <u>concentration</u> of the desired <u>buffer</u> anion, at the various pH's listed. These volumes and concentrations are given above each table. For each specie of buffer, two solutions are needed; a standard stock solution of some salt of the buffer anion, and a solution of either standard acid or alkali, usually NaOH or HCl. Instructions for making up the stock solutions are given above each table of buffers. A given volume of this stock is pipetted into a flask, the required amount of standard acid or alkali buretted into the same flask, and this mixture then diluted to the proper volume with distilled water, using a volumetric flask. This will give the listed volume of buffer at the specified anion concentration and at exactly the pH listed. If smaller or larger volumes are desired, the amounts of stock solution and standard alkali or acid should be altered proportionally. If an anion concentration other than that specified is desired, one alters the amounts of stock solution and standard acid or base proportionally, but does not change the total volume upon dilution. This will not significantly alter the pH, provided that the anion concentration is somewhere between 1/4 X and 2X that specified in the table. Within this range, the effects of dilution on the pH of buffers is negligible. In all cases, remember that the important factor is the ratio of stock anion solution to standard acid or base solution. As long as one maintains the proportions of these two solutions to each other, the buffer will have the proper pH.

In making up the stock anion solutions, it is always necessary to use the Merck Reagent Grade or Baker's Analyzed Grade chemical. If these bottles are initially opened in the dry room and kept in that room at low humidity at all times, the chemicals may generally be used directly without drying. For high accuracy, however, (i.e. in making standard buffers), the salts must be treated as directed and dried in an oven.

POTASSIUM CHLORIDE BUFFER: pH 1.0 - 2.2

Ref: Clark and Lubs: J. Biol. Chem; 25 479 (1916)

Stock Solution: 1.000M Potassium Chloride

Prepared by dissolving 14.912 g. of KCl in enough distilled water to make 200 ml. of solution. Merck's Reagent may be used directly if freshly opened in the dry room and kept there. For greater accuracy, dry four hours at 120°C.

Buffers:

To make 200 ml. of 0.05 M (1/20 M) buffer, pipette 10 ml. of the 1.000 M stock solution into a 200 ml. volumetric flask, add the volume standard (1.000M or 0.1000M as specified) HCl listed below, and dilute to the mark with distilled water.

pH	ml. 1.000M HCl	pH	ml. 0.1000M HCl
1.0	19.40	1.8	32.20
1.2	12.90	2.0	21.20
1.4	8.30	2.2	13.40
1.6	5.26		

POTASSIUM HYDROGEN PHTHALATE BUFFERS

With HCl: pH 2.2 - 3.8 With NaOH: pH 4.0 - 6.2

Ref: Clark and Lubs: J. Biol. Chem. 25 479 (1916)

Stock Solution: 0.200M Potassium Biphthalate

Prepared by dissolving 40.828 g. of Merck's Reagent Potassium Biphthalate in enough distilled water to make 1 liter. The Merck product may be used directly if opened and kept in the dry room. For greater accuracy, dry for several hours at 110°-120°C

Buffers:

To prepare 200 ml. of 0.05 M Phthalate buffer, pipette 50 ml. of the stock 0.200M solution into a 200 ml. volumetric flask, add the specified amount of the standard HCl or NaOH (1.000M or 0.1000M as listed) and dilute to the mark with distilled water.

pH	ml. of 1.000M HCl	pH	ml. of 0.1000M HCl
2.2	9.34	3.0	40.64
2.4	7.92	3.2	29.40
2.6	6.59	3.4	19.80
2.8	5.28	3.6	11.94
		3.8	5.26

pH	ml. of 0.1000M NaOH	pH	ml. of 1.000M NaOH
4.0	0.80	5.2	5.99
4.2	7.40	5.4	7.09
4.4	15.00	5.6	7.97
4.6	24.30	5.8	8.60
4.8	35.40	6.0	9.09
5.0	47.70	6.2	9.40

CITRATE BUFFERS

pH 2.2 - 6.0

Reference: Recalculated from Kolthoff and Vleeschhouwer; Biochem. Zeit. 183, 144 (1927) 179, 410 (1926)

Stock Solution: 0.500M Citric Acid

Weigh out 105.055 g. of dry, crystalline Citric Acid ($C_8H_8O_7 \cdot H_2O$) and dissolve in enough water to make 1 liter. Merck Reagent grade should be used, and it is satisfactory to use a bottle opened and kept in the dry room. To be sure of the molarity, titrate with 1.000M NaOH. The end-point is a distinct red color of the phenolphthalein indicator.

Buffers:

To make 250 ml. of 0.1M Citrate Buffer, pipette 50 ml. of the 0.500M stock into a 250 ml. volumetric flask. Add the number of ml. of 1.000N NaOH indicated below and dilute to the mark with distilled water.

pH	ml. 1.000NaOH	pH	ml. 1.000N NaOH
2.2	2.23	4.2	33.15
2.4	4.63	4.4	36.85
2.6	7.13	4.6	40.75
2.8	10.10	4.8	44.60
3.0	13.40	5.0	48.35
3.2	17.10	5.2	52.10
3.4	20.50	5.4	55.50
3.6	23.93	5.6	59.00
3.8	26.00	5.8	62.20
4.0	29.50	6.0	65.60

ACETATE BUFFERS

pH 3.6 - 5.6

Reference: Recalculated from Walpole, J. S., J. Chem. Soc. 105 2501 (1914)

Stock Solutions: 1.000N Acetic Acid
1.000N Sodium Hydroxide NaOH

Both of these solutions are prepared according to the section on standard solutions (CHAPTER V).

Buffers:

To make 1 liter of 0.1M Acetate Buffer, pipette 100 ml. of the 1.000N Acetic Acid stock solution into a 1 liter volumetric flask, burette in the specified amount of 1.000N NaOH, and dilute to the mark with distilled water.

pH	ml. 1.000N NaOH	pH	ml. 1.000N NaOH
3.6	7.5	4.8	60.0
3.8	12.0	5.0	70.5
4.0	18.0	5.2	79.0
4.2	26.5		
4.4	37.0	5.4	85.5
4.6	49.0	5.6	90.5

PHOSPHATE BUFFERS

pH 5.8 - 8.0

Reference: Recalculated from Clark and Lubs; J. Biol. Chem. 25 479 (1916)

Stock Solution: 1.000M Potassium Phosphate, Monobasic
(Potassium Dihydrogen Phosphate KH_2PO_4)

Prepared by dissolving 136.14 g. of KH_2PO_4 in enough distilled water to make 1 liter of solution. Merck's anhydrous reagent may be used directly if freshly opened in the dry room and kept there. For greater accuracy,dry for two hours at 120°C and place in a desiccator. The solid is conveniently weighed in 25 - 30 g. lots, using glazed weighing paper.

Buffers:

To make 1 liter of 0.1M Phosphate Buffer pipette 100 ml. of the 1.000M Phosphate stock solution into a 1 liter volumetric flask, add the volume of 1.000N NaOH specified below, and dilute to the mark with distilled water.

pH	ml. 1.000N NaOH	pH	ml. 1.000N NaOH
5.8	7.32	7.0	59.08
6.0	11.28	7.2	69.80
6.2	17.10	7.4	78.68
6.4	25.20	7.6	85.48
6.6	35.48	7.8	90.34
6.8	47.20	8.0	93.70

PHOSPHATE BUFFERS

pH 5.8 - 8.0

An alternate method of preparing phosphate buffers is to weigh out the proper proportion of dibasic and monobasic phosphate salts and dissolve to volume with distilled water. This procedure is of advantage under two circumstances:

a) When buffers in pyrex distilled water are desired, since this avoids the time-consuming procedure of preparing standard NaOH in pyrex distilled water.

b) When pure potassium or pure sodium phosphate buffers are desired, without the presence of the other ions. The advantage of the solids method is that no new standard solutions need be prepared.

To make 1 liter of M/15 Phosphate Buffer, weigh accurately the amount of monobasic phosphate (Na or K as desired) and the amount of dibasic phosphate (Na or K as desired) into a 1 liter volumetric flask, dissolve, and dilute to the mark with distilled water. Since dissolution may be slow, it is best, at first, to add 700 ml. of hot distilled water and shake until dissolved, then cool to room temperature and dilute to volume.

In each case, use Merck reagent grade salts of the formulas given below. Freshly opened chemicals, kept in the dry room, may be used without other drying.

PHOSPHATE BUFFERS M/15

Reference: Recalculated from Sorenson, S.P.L.; Ergeb. Physiol. 12 393 (1912)

Monobasic Salts			Dibasic Salts	
KH_2PO_4	$NaH_2PO_4 \cdot H_2O$	pH	K_2HPO_4	Na_2HPO_4
8.327g.	8.442g.	5.8	0.9580g.	0.7809g.
7.942	8.051	6.0	1.452	1.183
7.442	7.545	6.2	2.090	1.704

Monobasic Salts			Dibasic Salts	
KH_2PO_4	$NaH_2PO_4 \cdot H_2O$	pH	K_2HPO_4	Na_2HPO_4
6.648	6.739	6.4	3.106	2.532
5.695	5.773	6.6	4.326	3.526
4.583	4.646	6.8	5.748	4.685
3.540	3.588	7.0	7.083	5.774
2.587	2.622	7.2	8.303	7.768
1.770	1.794	7.4	9.348	7.620
1.248	1.265	7.6	10.015	8.164
0.7942	0.8051	7.8	10.596	8.637
0.4992	0.5060	8.0	10.973	8.945

Mix the monobasic and dibasic salts, dissolve in 700 ml. of hot distilled water, cool to room temperature, and dilute to 1 liter in a volumetric flask.

BARBITAL (VERONAL) BUFFER

pH 6.8 - 9.2

Reference: Recalculated from J.B.C. 87 33 (1930)

Stock Solution: 0.500M Sodium Veronal (also called Disodium Barbital, Barbital Soluble)

Prepared by weighing out 51.545 g. of Veronal Sodium (Merck Powder) and dissolving in enough distilled water to make 500 ml. The powder, as it comes in the bottle, may be used directly without drying.

Buffers:

To make 500 ml. of 0.1M Veronal Buffer pipette 100 ml. of the 0.500M stock solution into a 500 ml. volumetric flask, add the volume of 1.000M HCl specified below, and dilute to the mark with distilled water.

†pH	ml. 1.000N HCl	pH	ml. 1.000N HCl
6.8	45.80	8.2	15.05
7.0	43.30	8.4	10.75
7.2	40.25	8.6	7.40
7.4	36.05	8.8	5.07
7.6	31.30	9.0	3.42*
7.8	25.53	9.2	2.52*
8.0	19.85		

†It is difficult to prepare buffers of a pH lower than 8.8. This is due to the ppt. of barbituric acid at low pHs.

*Here, for better accuracy, ten times as much 0.1000N HCl may be used instead.

GLYCYLGLYCINE BUFFERS

pH 7.6 - 9.0

Reference: Determined experimentally in M.B.L. Chemical Room by J. D. Ostrow and J. B. Russell, 1951. Data available on request.

Stock Solution: 0.500M Glycylglycine

Weigh accurately 33.030 g. of glycylglycine into a 500 ml. volumetric flask and dilute to the mark with distilled water. Use high purity chemical directly from a bottle opened and kept in the dry room.

Buffers:

To make 250 ml. of 0.1M buffer pipette 50 ml. of the 0.500M stock solution into a 250 ml. volumetric flask. Add the volume of 1.000N NaOH indicated in the table below, and dilute to the mark with distilled water.

pH	ml. 1.000N NaOH	pH	ml. 1.000N NaOH
7.6	4.40	8.4	14.63
7.8	6.35	8.6	17.46
8.0	9.10	8.8	19.52
8.2	11.85	9.0	21.10

Note: A charge will be made for all glycylglycine buffers due to the expense of this chemical.

BORATE-KCl-NaOH BUFFERS

pH 7.8 - 10.0

Reference: Clark and Lubs; J. Biol. Chem. 25 479 (1916)

Stock Solution: A mixture of 0.500M Boric Acid H_3BO_3 and 0.500M Potassium Chloride KCl

Weigh out 31.0120 g. Boric Acid and 37.280 g. of KCl and dissolve in enough distilled water to make 1 liter of solution. Use Merck's Reagent grade chemicals. The solid may be weighed directly from the bottle, without previous drying, if freshly opened and kept in the dry room. For better accuracy, however, the KCl should be dried at 120°C in an oven for four hours, and the Boric Acid dried in thin layers over $CaCl_2$ in a desiccator. Under no circumstances should the Boric Acid be heated above 50°C, or it loses water of constitution forming metaboric acid HBO_2.

Buffers:

To make 1 liter of 0.05M buffer, pipette 100.0 ml. of the 0.5M stock solution into a 1 liter volumetric flask, add the volume of 1.000N NaOH specified below, and dilute to the mark with distilled water.

pH	ml. 1.000N NaOH	pH	ml. 1.000N NaOH
7.8	2.65	9.0	21.40
8.0	4.00	9.2	26.70
8.2	5.90	9.4	32.00
8.4	8.55	9.6	36.85
8.6	12.00	9.8	40.80
8.8	16.40	10.0	43.90

CARBONATE-BICARBONATE BUFFERS
pH 9.4 - 10.4

Reference: Determined in M.B.L. Chemical Room by J. D. Ostrow and J. B. Russell, 1951. Data available on request.

Stock Solution: 0.500M Sodium Bicarbonate

Weigh accurately 21.0050 g. $NaHCO_3$ into a 500 ml. volumetric flask and dissolve to the mark with distilled water. Use Merck's reagent grade. The material may be used without drying if freshly opened in the dry room, but for more accuracy, dry at 110°C for four hours and store in a desiccator.

Buffers:

To make 250 ml. of 0.1M Carbonate buffer, pipette 50ml. of the 0.500M Bicarbonate stock solution into a 250 ml. volumetric flask, burette in the volume of 1.000N NaOH indicated in the table below, and dilute to volume with distilled water.

pH	ml. 1.000N NaOH	pH	ml. 1.000N NaOH
9.4	4.85	10.0	13.73
9.6	7.63	10.2	16.78
9.8	10.70	10.4	19.58

HIGH-pH PHOSPHATE BUFFER
pH 11.0-12.0

Reference: Biochem. Zeitschr. 189 191 (1927);Kolthoff and Vleeschhouwer

Stock Solution: 0.500M Na_2HPO_4 .

Weigh out accurately 71.01 g. and dilute to 1 liter in a volumetric flask. Freshly opened reagent grade chemicals may be used if kept in the dry room. For greater accuracy, dry in an oven for two hours at 120°C.

Buffers:

To prepare 250 ml. of 0.1M Phosphate buffer pipette 50 ml. of the 0.500M stock secondary phosphate solution into a 250 ml. volumetric flask, add the volume of 1.000N NaOH indicated on the table below, and dilute to the mark with distilled water.

pH	ml. 1.000N NaOH	pH	ml. 1.000N NaOH
11.0	4.13	11.6	12.25
11.2	6.00	11.8	16.65
11.4	8.67	12.0	21.60

McILVAINE BUFFERS

pH 2.2 - 8.0

Reference: J. Biol. Chem. 49 183 (1921)

Stock Solutions:

A) 0.500M Citric Acid:

Weigh out 105.055 g. of dry Citric Acid ($C_6H_8O_7 \cdot H_2O$) and dissolve in enough water to make 1 liter. The Merck reagent grade, if taken from a bottle freshly opened in the dry room, is satisfactory. To be sure of the molarity, standardize with 1.000M NaOH. The titration is carried to a distinct red color of the phenolphthalein indicator.

B) 0.500M Na_2HPO_4:

Weigh out 71.01 g. accurately and dilute to 1 liter with distilled water in a volumetric flask. Freshly opened Merck Reagent or Baker's analyzed grade chemicals may be used if kept in the dry room, but for greater accuracy, dry in an oven for two hours at 120°C and place in a desiccator.

Buffers:

To prepare 200 ml. of buffer, burette the amounts of stock solutions indicated below into a 200 ml. volumetric flask, and dilute to the mark with distilled water.

pH	ml. 0.500M Na_2HPO_4	ml. 0.500M Citric Acid
2.2	1.60	39.20
2.4	4.96	37.52
2.6	8.72	35.64
2.8	12.68	33.66
3.0	16.44	31.78
3.2	19.76	30.12
3.4	22.80	28.60
3.6	25.95	27.12
3.8	28.40	25.80
4.0	30.84	24.58
4.2	33.12	23.44

pH	ml. 0.500M Na_2HPO_4	ml. 0.500M Citric Acid
4.4	35.28	22.36
4.6	37.40	21.30
4.8	39.44	20.28
5.0	41.20	19.40
5.2	42.88	18.56
5.4	44.60	17.70
5.6	46.40	16.80
5.8	48.36	15.82
6.0	50.52	14.74
6.2	52.88	13.56
6.4	55.40	12.30
6.6	58.20	10.90
6.8	61.80	9.10
7.0	65.88	7.06
7.2	69.56	5.22
7.4	72.68	3.66
7.6	74.92	2.54
7.8	76.60	1.70
8.0	77.80	1.10

UNIVERSAL BUFFER (TEORELL)

pH 2.0 - 12.0

Reference: Teorell, Tand and Stenhagen, Biochem. Zeitschr
299 416-9 (1938)

General:

This buffer contains the buffer anions phosphate, borate, and citrate. It covers the pH's 2 - 12, and has a practically linear buffer capacity in the range pH 3 - 11. Between these values, 5 ml. of 0.1N acid or base shifts the pH about 1 unit. The molarities of the various ions in the final buffers are as follows:

Phosphate - M/100	Borate - M/100
Citrate - M/150	Sodium - M/15

Standard Solutions:

1) 1.000N NaOH...See section on standards.

2) 0.1000N HCl...See section on standards.

3) 1N Phosphoric Acid (H_3PO_4)...Dilute 17.5 ml. of 85% highest grade H_3PO_4 to 500 ml. Standardize by titrating against 20 ml. aliquots of the standard NaOH, using phenolphthalein indicator. The end-point is taken as last trace of rose color, after the initial bright red has lightened

into the rose tint. Calculate the ml.'s of the acid equivalent to 100 ml. of the NaOH.

4) 1N Citric Acid. ($H_3C_6H_5O_7 \cdot H_2O$)...Dissolve 35 g. of the Merck reagent crystals in enough distilled water to make 500 ml. Standardize against the 1.000N NaOH exactly as with the phosphoric acid, taking the same end-point. Store in the refrigerator.

5) Boric Acid Crystals. (H_3BO_3)...Dry Merck's reagent grade in thin layers over $CaCl_2$ in a desiccator.

Stock Solution:

Using a burette, measure into a 1 liter volumetric flask the volumes of phosphoric acid solution (3) and citric acid solution (4) equivalent to 100.0 ml. of the 1N NaOH, as determined by the titrations. Add 343.0 ml. of 1N NaOH and 3.54 g. of the Boric Acid and dilute to the mark with distilled water.

Buffers:

To make 100 ml. of buffer at the ionic concentrations given above, pipette 20 ml. of stock solution into a 100 ml. volumetric flask, add the specified volume of 0.1000N HCl as given in the table below, and dilute to the mark with distilled water.

	ml. of 0.1000N HCl									
pH	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
2	73.30	70.35	67.85	65.70	63.85	62.25	60.80	59.55	58.45	57.40
3	56.50	55.70	54.95	54.30	53.70	53.20	52.65	52.10	51.55	51.02
4	50.50	49.97	49.45	48.91	48.35	47.80	47.26	46.75	46.22	45.68
5	45.18	44.60	44.05	43.50	42.94	42.36	41.80	41.23	40.61	40.00
6	39.42	38.94	38.09	37.45	36.74	36.06	35.36	34.65	33.92	33.25
7	32.65	31.98	31.45	30.83	30.35	29.87	29.43	29.05	28.68	28.33
8	28.02	27.69	27.45	27.25	26.90	26.60	26.10	25.63	24.90	24.33
9	23.75	23.05	22.38	21.72	21.12	20.52	19.94	19.37	18.81	18.35
10	17.92	17.43	16.97	16.64	16.36	16.15	15.95	15.70	15.40	15.02
11	14.52	13.93	13.20	12.30	11.23	10.00	8.40	6.60	4.70	2.06
12	0.40									

CHAPTER VIII.

SALINE AND ARTIFICIAL SEA WATER SOLUTIONS

SALINES AND SEA-WATERS: GENERAL

Artificial salt solutions have been prepared which attempt to duplicate both the osmotic and ionic properties of the internal or external environments of given organisms. The aquatic media in which animals live are replaced by artificial sea-waters, while the Ringer's and associated solutions are substitutes for the tissue fluids (and/or plasma) of the various species. In many cases, it suffices to use a solution of a single compound which has the same osmotic pressure as the medium, a so-called isotonic solution. Such solutions, though osmotically normal, are physiologically imperfect due to ionic imbalance.

The chief components of these artificial salines are sodium, potassium, calcium, and magnesium cations, and the anions chloride sulfate, and bicarbonate. They are readily added in the form of six salts mixed in the proper proportions, and at the proper total concentration. Reagent Grade Merck chemicals and distilled water should be used in all cases. Merck's special reagent for biological use sodium chloride should be used to avoid toxic impurities sometimes found in other brands. The bicarbonate must not be added until the solution is almost diluted to volume, nor before heat sterilization (if necessary) or the calcium will precipitate.

SALT STANDARDS:

For convenience in making up the artificial sea-waters and physiological salt solutions, 8 liter stock bottles of 1.00M solutions of the major components are kept above the Chemist's work bench. These are connected to self-filling burettes for ready delivery of the proper volumes of the standard salt solutions.

Sodium chloride, potassium chloride, sodium bicarbonate, and magnesium sulfate are not hygroscopic, and their solutions may be prepared by weighing out the proper amount of the salt as it comes in the bottle, and dissolving to volume. Calcium chloride and magnesium chloride are too hygroscopic for this procedure, and therefore approximate amounts of these salts are dissolved to volume and the resulting solutions standardized and adjusted to 1.00M.

The standardization is done by determining the chloride content,

rather than by the difficult analyses of the respective cations. 10 ml. of the unknown is pipetted into a 125 ml. erlenmeyer, diluted to 50 ml., and titrated with standard silver nitrate (about 1 molar), using three drops of dichlorofluorescein indicator. The titration must be done as rapidly as possible to avoid masking of the end-point by the purple color of reduced silver. The end-point is taken as the first persistent salmon-pink color in the mixture.

The indicator is prepared by dissolving 0.1 g. of dichlorofluorescein and 2.5 ml. of 0.1N NaOH in 100 ml. of distilled water, and does not work in acid solutions below pH 5.5. The silver nitrate is standardized by titration against accurately weighed 1.1-1.2 g. samples of reagent sodium chloride. The NaCl is placed in a 125 ml. erlenmeyer, dissolved in 50 ml. of distilled water, and titrated by the same method as is used on the unknowns. It will take about 20 ml. of silver nitrate to titrate NaCl samples of the recommended amount.

Weight:Volume Factors for Salt Standards

1.000 Molar	gms./ml.	ml./gram
NaCl	0.05845	17.11
KCl	0.07456	13.42
$CaCl_2 \cdot 2H_2O$	0.14703	6.81
$MgCl_2 \cdot 6H_2O$	0.20333	4.92
$MgSO_4 \cdot 7H_2O$	0.24649	4.06
$NaHCO_3$	0.08402	11.91

Solutions Isotonic with Sea Water Salinity 35 $^{0}/_{00}$

NaCl	0.53M
KCl	0.53M
$CaCl_2 \cdot 2H_2O$	0.34M
$MgCl_2 \cdot 6H_2O$	0.37M
$MgSO_4 \cdot 7H_2O$	0.90M
$NaHCO_3$	0.54M
NaBr	0.54M
$Na_2SO_4 + 10H_2O$	0.44M
CsCl	0.53M
RbCl	0.58M
LiCl	0.60M
$C_{12}H_{22}O_{11}$	0.81M

Challenger Report on Sea Water:	gm/l
NaCl	27.213
$MgCl_2$	3.807
$MgSO_4$	1.658
$CaSO_4$	1.260
K_2SO_4	0.863
$CaCO_3$	0.123
$MgBr_2$	0.076
HBr	0.06

For Woods Hole dilute 30.8 volumes to 35 volumes.

Analysis of Sea Water at Woods Hole: (Page, 1927)	gm/l
Na	8.80
K	0.412
Ca	0.428
Mg	1.3004
Cl	18.350
SO_4	2.615
PO_4	0.002

Cation Analysis of Woods Hole Sea Water (Shanklin, 1954)	Millimols Per Litre
Na	534.0
K	18.2
Mg	56.2
Ca	5.8

ELEMENTS PRESENT IN SOLUTION IN OCEANIC SEA WATER EXCLUSIVE OF DISSOLVED GASES*

Chlorinity = 19.00 0/00

Element	Parts per Million	Element	Parts per Million
Chlorine	18980	Copper	0.001-0.01
Sodium	10561	Zinc	0.005
Magnesium	1272	Lead	0.004
Sulfur	884	Selenium	0.004
Calcium	400	Cesium	0.002
Potassium	380	Uranium	0.0015
Bromine	65	Molybdenum	0.0005
Carbon	28	Thorium	0.0005
Strontium	13	Cerium	0.0004
Boron	4.6	Silver	0.0003
Silicon	0.02-4.0	Vanadium	0.0003
Fluorine	1.4	Lanthanum	0.0003
Nitrogen†	0.006-0.7	Yttrium	0.0003
Aluminum	0.5	Nickel	0.0001
Rubidium	0.2	Scandium	0.00004
Lithium	0.1	Mercury	0.00003
Phosphorus	0.001-0.10	Gold	0.000006
Barium	0.05	Radium	$0.2\text{-}3 \times 10^{-10}$
Iodine	0.05	Cadmium	present
Arsenic	0.002-0.02	Cobalt	present
Manganese	0.001-0.01	Tin	present

* H. U. Sverdrup, M. W. Johnson, and R. H. Fleming, The Oceans, Prentice-Hall, Inc., New York, 1942.

† Nitrogen in combined forms.

ARTIFICIAL SEA WATERS:

Sea waters all over the world contain the same proportions of the various ions, differing only in the total ionic content and therefore in total osmotic pressure. The osmotic pressure is proportional to the salinity of the sea water, this being defined as the total grams of solid per kilogram of sea water when all the halides have been converted to chloride, all the carbonate converted to oxide, and all the organic matter completely oxidized. The salinity of Woods Hole sea water is 31 0/00±0.5. All the formulae given below have been recalculated to the same salinity, and therefore the same osmotic pressure, as Woods Hole sea water.

A true artificial Woods Hole sea-water, called M.B.L. formula, is listed in the table below. This contains all the major ions in amounts identical with Woods Hole sea water as it comes from the sea water taps, except that the fluorides, bromides and iodides have been replaced by chloride, the strontium replaced by calcium, and the borate omitted. In the Trace M.B.L. Formula, however, these minor elements are included in their proper amounts. These formulae have been tested on sea urchin eggs by Ethel Browne Harvey and have been found to be both osmotically and physiologically satisfactory. They are the recommended formulae for use at the M.B.L.

The other listed formulas are experimental sea waters which were used by the investigators whose names are applied to the solutions. They all differ from sea water in all components, but contain approximately the same amounts of the various ions as sea water (compare with M.B.L. formula). The Allen (Pantin) and Brujewicz formulae correspond most closely to true sea water, all the others being low in sulfate and equivalently higher in chloride. The Van't Hoff formulae are low in calcium and lack the buffer action of bicarbonate, whereas, the Challenger sea water contains over double the normal amount of calcium. The two calcium-free formulae both replace the calcium with sodium so as to maintain osmotic pressure, but the Shapiro formula is also low in magnesium.

The listed quantities of the various salts are mixed in a 1 liter volumetric flask and diluted to the mark with distilled water. It is best to weigh out the sodium chloride, but to burette the 1.00M solutions for all the other components. Ml. of 1.00M refers to milliliters of 1.00M solution of the salt. Be sure to check the notes below the main table for any minor constituents which must be added before diluting to volume.

TABLE OF ARTIFICIAL SEA WATERS

All recalculated to salinity of 31 0/00 and osmotically identical with Woods Hole sea water.

AUTHOR	$NaCl$ ml. 1.00M	g/l	KCl ml. 1.00M	g/l	$CaCl_2 \cdot 2H_2O$ ml. 1.00M	g/l	$MgCl_2 \cdot 6H_2O$ ml. 1.00M	g/l	$MgSO_4 \cdot 7H_2O$ ml. 1.00M	g/l	$NaHCO_3$ ml. 1.00M	g/l
Allen (same as Pantin)*	423.0	24.72	9.10	0.68	9.50	1.40	23.50	4.78	25.53	6.30	1.15	0.097
Brujewicz (1)	419.9	24.51	9.00	0.67	9.50	1.40	23.75	4.82	25.38	6.26	2.23	0.187
Challenger (2)	395.0	23.10	8.88	0.66	21.15	3.12	31.36	6.36	16.78	4.14	xxxx	xxxxx
Horstadius-Bialascewicz (3)	430.5	25.17	8.73	0.65	9.76	1.44	28.36	5.78	18.35	4.53	2.18	0.183
Horstadus-Roscoff (3)	428.0	25.02	9.80	0.73	9.97	1.47	29.35	5.96	18.07	4.47	2.38	0.200
M.B.L. Formula (4)	423.0	24.72	9.00	0.67	9.27	1.36	22.94	4.66	25.50	6.29	2.15	0.180
Moore Calcium-free #	436.0	25.48	9.68	0.72	xxxxx	xxxx	34.13	6.94	16.67	4.11	xxxx	xxxxx
Shapiro Calcium-free #	442.5	25.88	9.83	0.74	xxxxx	xxxx	24.58	5.00	16.80	4.14	xxxx	xxxxx
Trace M.B.L. Formula (5)	423.0	24.72	8.27	0.62	9.27	1.36	22.94	4.66	25.50	6.29	2.15	0.180
Van't Hoff alpha (6)	430.5	25.17	9.40	0.70	7.22	1.06	34.31	6.96	16.52	4.07	trace	
Van't Hoff beta (6)	432.0	25.23	9.54	0.71	4.36	0.64	33.78	6.86	16.52	4.07	xxxx	xxxxx

Notes and References for Table Above:

*) No references found except for Formulae and Methods III.

1) Subow N.N.: Oceanographic Tables, Moscow (1931) * Add NaBr 0.077 g/l
2) Adapted from the Challenger Report - Add Na_2CO_3 1.16 g/l and $MgBr_2 \cdot 6H_2O$ 0.11 g/l
3) Publ. Staz. Zool. Napoli 14 253-429 (1935)
4) Calculated by J.D. Ostrow from Table of Major Constituents of Sea Water, Lyman & Fleming, J. Marine Research 3 134-146 (1940). Tested by Ethel Browne Harvey, M.B.L. 1951.
5) Calculated by J.D. Ostrow from Table of Major Constituents of Sea Water, Lyman & Fleming, J. Mar. Res. 3 134-146 (1940). Add KBr 0.089g.; NaF 0.003g.; $SrCl_2 \cdot 6H_2O$ 0.037g.; and H_3BO_3 0.024g. per liter of solution.
6) Original reference for both forms is Van't Hoff, J.H., Physical Chemistry in the Service of the Sciences, p. 101. Univ. of Chicago Press (1903); alpha formula from C.G. Rogers Textbook of Comparative Physiology (1927) N.Y.; beta formula from Osterhout. W.J.V., Bot. Gaz., 42 127 (1906)

PHYSIOLOGICAL SALINE SOLUTIONS

In working with isolated tissues, it is important to bathe the preparation in balanced saline solutions which duplicate the ionic composition of the tissue fluids and plasma of the donor animal. Such solutions are known as Ringer's solutions, after the man who first stressed the importance of ions in physiological function. The following table is a completely revised list of balanced salt solutions, including the most recent formulae available.

The Boyle-Conway Amphibian Ringer and the Krebs Mammalian Ringer are recommended by Dr. Szent-Gyoergyi. They are almost identical in every respect with the plasma of those two classes, except for the protein components, which are not included in the synthetic media. These will be our stock Ringers for amphibians and mammals. The Locke Mammal Ringer is the old, standard formula for work on mammalian hearts, whereas the Tyrode Mammal Ringer is better for gut muscle. Two Molluscan Ringers are included, one for marine animals (determined for mussel) and one for fresh water animals (determined for Helix).

Four other solutions of a Ringer type are listed. They are given in Formulae and Methods III, but original literature could not be located. Be sure to check notes below table for other components.

PHYSIOLOGICAL SALINE SOLUTIONS

RINGER'S SOLUTIONS	NaCl ml. 1.00M	NaCl g/l	KCl ml. 1.00M	KCl g/l	$CaCl_2 \cdot 2H_2O$ ml. 1.00M	$CaCl_2 \cdot 2H_2O$ g/l	$MgCl_2 \cdot 6H_2O$ ml. 1.00M	$MgCl_2 \cdot 6H_2O$ g/l	$NaHCO_3$ ml. 1.00M	$NaHCO_3$ g/l	Dextrose g/l
Amphibian (regular) (1)	111.2	6.50	1.88	0.14	1.08	0.16	xxxxx	xxxx	2.38	0.20	xxx
Amphibian (Boyle-Conway-2)	72.6	4.24	1.99	0.15	xxxx	xxxx	xxxxx	xxxx	25.01	2.10	xxx
Crayfish (3)	205.3	12.00	5.37	0.40	13.55	1.99	2.61	0.53	2.38	0.20	xxx
Crustacean (4)	525.0	30.65	13.27	0.99	12.39	1.82	24.78	5.04	to pH	7.0	xxx
Elasmobranch (5)	280.2	16.38	11.94	0.89	10.00	1.47	xxxx	xxxx	4.52	0.38	1.00
Insect (6)	154.0	9.00	2.68	0.20	1.84	0.27	xxxx	xxxx	to pH	7.2	4.00
Mammal (Krebs) (7)	118.4	6.92	4.70	0.35	2.52	0.37	xxxx	xxxx	25.01	2.10	xxx
Mammal (Locke) (8)	154.0	9.00	5.64	0.42	2.16	0.32	xxxx	xxxx	2.38	0.20	2.00
Mammal (Tyrode) (9)	136.9	8.00	2.68	0.20	1.84	0.27	1.03	0.21	11.91	1.00	1.00
Molluscan (Marine) (10)	530.0	30.97	10.70	0.80	13.00	1.91	xxxx	xxxx	xxxx	xxxx	xxx
Molluscan (Fresh-water:11)	98.2	5.74	11.94	0.89	17.97	2.64	27.64	5.62	24.42	2.05	xxx
Other Solutions*											
Belar's	154.0	9.00	2.68	0.20	1.84	0.27	xxxx	xxxx	2.38	0.20	4.00
Clark's (12)	111.2	6.50	1.88	0.14	1.08	0.16	xxxx	xxxx	1.19	0.10	xxx
Holtfreter's	59.9	3.50	0.67	0.05	0.90	0.10	xxxx	xxxx	2.38	0.20	xxx
Knowlton's (13)	220.0	12.90	6.97	0.52	3.96	0.58	5.05	1.02	xxxx	xxxx	xxx

Notes and References for Table of Physiological Salines

*) No reference found except Formulae and MethodsIII.

1) Winton and Bayliss; Human Physiology, p. 393, 2nd Ed. Blakiston (1935). Add $NaH_2PO_4 \cdot H_2O$ 0.012 g/l
2) Boyle and Conway; J. Physiol., 100, 1 (1941). Add $MgSO_4 \cdot 7H_2O$ 1.22 ml. of 1M; $Na_2SO_4 \cdot 10H_2O$ 0.21 g/l. Just before use add - Na_2HPO_4 0.36 g.; KH_2PO_4 0.07 g.; Calcium Gluconate 0.40 g.
3) Harreveld, A. V.; Proc. Soc. Exper. Biol. & Med., 34, 428 (1936).
4) Pantin; J. Exper. Biol., 11, 11 (1934).
5) Babkin, B.P. et al.; Contr. Canad. Biol. & Fish N.S., 8, 209 (1933). Add urea 21.6 g.; $NaH_2PO_4 \cdot H_2O$ 0.07 g.
6) Pringle, J.W.S.; J. Exper. Biol., 15, 144 (1938).
7) Krebs, A.; Hoppe-Seyler's Zeitschr., 210, 33 (1932). Add $MgSO_4 \cdot 7H_2O$ 1.18 ml. of 1M; $KH_2PO_4$0.16 g/l
8) Locke & Rosenheim; J. Physiol., 36, 208 (1907).
9) Gellhorn, E.; Lehrbuch der Allgem. Physiol, p. 74, Thieme (Leipzig 1931). Add $NaH_2PO_4 \cdot H_2O$ 0.06 g/l.
10) Singh, I.; J. Physiol. 92, 62 (1938). Add phosphate buffer at pH 7.2 to strength desired.
11) Bernard and Bonnet; C.R. Soc. Biol. Paris, 103, 1119 (1930).
12) No reference. Add $NaH_2PO_4 \cdot H_2O$ 0.012 g/l
13) No reference. Add urea 20.0 g. Used for perfusing dogfish heart.

CHAPTER IX.

PHOTOGRAPHIC SOLUTIONS

General Information:

Stock bottles of all the basic photographic solutions are kept in the Chemical Room, from which they may be issued to investigators. Only the formulas listed in this manual are kept in stock, all others being of a more specialized nature and prepared by the Chemist on order.

Photo solutions are generally unstable to air and deteriorate on standing if left exposed to the atmosphere. They are also unstable to light. For these reasons, they are always stored and issued in brown-glass bottles, which are initially filled to the top. Orders are drawn from already opened bottles before a fresh, completely-filled bottle is opened. As a guide to the investigator, a table of the keeping properties of photo solutions is included in this manual.

Preparation of Solutions - General:

The following list includes all the chemicals necessary for the preparation of the formulas in this manual. In every case, photo or technical grade materials are satisfactory, but distilled water should be used in making all solutions. All formulas are recalculated from those given in the Kodak Reference Handbook.

List of Photo Chemicals

Elon (Kodak) or Metol (Ansco)
or Photol (Merck)
Sodium sulfite, anhydrous
Hydroquinone
Kodalk (E.K.Co.)
Sodium thiocyanate
Sodium carbonate, monohydrate, $Na_2CO_3 \cdot H_2O$
Potassium Bromide, KBr
Borax (granular)
Boric acid crystals

Potassium alum
Chrome alum
Glacial acetic acid
Conc. sulfuric acid
Sodium sulfate
Sodium thiosulfate, (rice cryst., Hypo)
Potassium ferricyanide
Potassium dichromate

Preparation of Developers:

The six stock developers and their uses are listed in the table below:

D-11...High contrast for films and plates

D-19...Rapid contrast for films and plates
DK-20..Fine grain for films and plates
DK-50..General and Professional use on films and plates
D-72...General use for films, plates and papers
D-76...Maximum speed with normal contrast and
good shadow detail on films and plates

Note: ''Microdol'' is DK-20 with preservative added.
''Dektol'' is D-72 with preservative added.

Developers are prepared in 8-liter batches. For this purpose, an automatic stirrer and a graduated 8-liter jug (with pouring lip) are provided for the use of the Chemist. The warm water is heated to about 50°C in a 4-liter beaker over a Fischer burner, and then placed in the jug. The stirrer is set in motion and the weighed chemicals added in the order listed, each constituent being added only after the previous one has dissolved. When all the chemicals are in solution, cold water is added to make 8 liters. The inaccuracy in judging this from the graduation mark on the side of this jug is not crucial. The solution is then poured into the stock bottles with the aid of a large funnel provided for this purpose.

Ingredients	D-11	D-19	DK-20	DK-50	D-72	D-76
Distilled Water (50°C)	4 L	4 L	6 L	4 L	4 L	6 L
Elon, Metol, or Photol	8.0 g	17.6 g	40.0 g	20.0 g	24.8 g	16.0 g
Sodium Sulfite, anhydrous	600 g	768 g	800 g	240 g	360 g	800 g
Hydroquinone	72.0 g	70.4 g	xxxx	20.0 g	96.0 g	40.0 g
Kodalk	xxxx	xxxx	16.0 g	80.0 g	xxxx	xxxx
Sodium Thiocyanate	xxxx	xxxx	8.0 g	xxxx	xxxx	xxxx
Sodium Carbonate, Monohydrate	234 g	449 g	xxxx	xxxx	632 g	xxxx
Potassium Bromide	40.0 g	40.0 g	4.0 g	4.0 g	15.2 g	xxxx
Borax (granular)	xxxx	xxxx	xxxx	xxxx	xxxx	16.0 g

Dissolve completely and dilute to 8 liters with cold distilled water.

Preparation of Acid Fixing Bath with Hardener F-5:

This is the only acid fix kept in stock since it is the general purpose fixing bath, suitable for films, papers, and plates. For reasons of stability, it is stored as two solutions which are mixed to order. One is a 30% Hypo (Sodium Thiosulfate) solution and the other is the Hardener F-5A. Both are kept in bottles on the pressure system, and mixed in a graduated cylinder in the ratio 1 part Hardener to 4 parts Hypo. Solutions must be cool before mixing.

Preparation of Hardener F-5A: (8 liter batch)

This is mixed in the same jug with the same stirrer as was used on the developers. Ingredients are added in the order listed below:

1. Distilled water (50°C)..............4800 cc.
2. Sodium sulfite, anhydrous........... 600 g.
3. Acetic acid, glacial................ 513 cc.
4. Boric acid crystals................. 300 g.
5. Potassium alum...................... 600 g.
6. Cold water to make.................. 8 l.

Preparation of 30% Hypo:

If making a 12 liter batch use 3600g of the Hypo(Sodium Thiosulfate), if making an 18 liter batch use 5400g of the Hypo. By dissolving the Hypo in separate batches the crystals may be made to go into solution more easily and more quickly. Therefore, if making a 12 liter batch use two portions of distilled water of 6 liters each into which are dissolved 1816g (4lbs.) and 1784g (3.9lbs.) respectively. If making an 18 liter batch it is best to dissolve the Hypo in three separate 6 liter batches with 1816g (4lbs.) of the Hypo in two of the three batches and 1784g (3.9lbs.) in the third batch. It is easiest and more convenient to pour the one pound boxes of the Hypo directly into the stirring jug assuming them to be fairly accurate pounds. Any colloidal cloudiness will clear up in a day or two while in the carboy. However, the Hypo can be used even though it is cloudy. The cloudiness does not affect its effectiveness. Each 6 liter portion is poured into the carboy and then the carboy is placed under the pressure system beside the hardener.

Preparation of Stop and Hardening Baths:

1. Stop Bath SB-1: (1.3% Acetic acid)
   For papers and plates. 13 cc. of glacial acetic acid in 1 liter of water.
2. Hardening Bath SB-3: (3% Chrome alum)
   For use at summer room temperatures with films and plates. 30 g. of chrome alum dissolved in 1 liter of water.
3. Stop Bath SB-5: (Non-swelling acid rinse for Photo finishing)
   Water....................500 cc.
   Acetic acid, glacial..... 9 cc.
   Sodium sulfate crystals..105 g.
   Water to make............1.0 l

Miscellaneous Formulae:

Farmer's Reducer R-4A: (Cutting - for clearing shadow areas)

Stock Solution:

Dissolve 37.5 g. potassium ferricyanide in enough water to make 500 cc. For use, take 30 cc. of stock, 96 cc. of 30% Hypo, and enough water to make 1 liter. Mix the stock and hypo, then add the water and pour the mixed solution at once over the negative to be reduced. When sufficient reduction has occurred, remove the negative and wash thoroughly. The mixed reducer will keep only briefly.

General Tray Cleaner TC-1:

Dissolve 82 g. of potassium dichromate in 910 cc of hot water. Cool, and then add slowly with constant cooling and stirring, 87 cc. of conc. sulfuric acid.

Keeping Properties of Solutions:

The figures given are for no loss in quality on standing without use.

Developers except D-72:

1. In tray - 24 hours.
2. In gallon tank - 1 month.
3. In stoppered bottle, full - 6 months.
   half-full - 2 months.

D-72:

1. Tray - 24 hours.
2. Gallon tank - 2 weeks.
3. Stoppered bottle, full - 3 months.
   half-full - 1 month

Stop-Baths: (SB-1, 3, 5)

1. Tray - 3 days.
2. Tank - 1 month
3. Stoppered bottle - indefinitely

Acid-Fixing Bath F-5:

1. Tray - 1 week
2. Gallon tank - 1 month
3. Stoppered bottle, full - 3 months.
   half-full - 2 weeks

Useful Life of Photo Solutions:

This table is given in terms of the number of 8 X 10 inch sheets which can be processed per gallon of developer without impairment of the quality of the solution.

Solution	8" X 10" sheets/gal. Tray	Deep Tank
Developer D-11	20	40
Developer D-19	30	60
Developer DK-20	20	30
Developer DK-50	20	40
Developer D-72	20 (1:1)	40 (1:1) Neg.
	15 (1:2)	30 (1:2) Neg.
	30 (1:1) Prints	
	30 (1:2) Prints	
	25 (1:4) Prints	
Developer D-76	20	30
Acid Fixing Bath F-5	100	100
Stop Bath SB-1	75	75
Hardening Bath SB-3	25	25
Stop Bath SB-5	100	100